Phenotypic diversity of Spanish apple (*Malus x domestica* Borkh) accessions grown at the vulnerable climatic conditions of the Ebro Valley, Spain

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Abstract

Agronomic, morphological and fruit quality traits were studied for 80 accessions from an apple Germplasm Bank established at the Experimental Station of Aula Dei, located in the central part of the Ebro Valley (Northeastern Spain, Zaragoza). This area is characterized by low-medium altitude, warm summers with hot day-night temperatures, cold winters, and low annual precipitation. Most accessions were surveyed during the last decades from Spanish regions with different climate environments. Agronomic traits included bloom and harvest dates, while morphological traits included different fruit shape and size characteristics. Fruit quality parameters included percentage of over color, chromaticity parameters (L^* , a^* , b^* , C^* and h^*), flesh firmness, soluble solids content and titratable acidity. Extensive variability was observed for most traits with significant correlations identified between many traits. Principal component analysis (PCA) revealed the main sources of variability in the set of accessions evaluated. Based on these sources, the cluster analysis produced six different groups of accessions according to the Ward's minimum variance criterion. Eight groups of synonymies were founded, which represented 21% of the total accessions studied. On the contrary, some accessions with the same allelic profile exhibit phenotypic differences, suggesting the existence of clonal mutations not detected with molecular markers. This study provides useful information for agronomic, morphological and fruit quality traits from a wide range of accessions grown under the Ebro Valley vulnerable climatic conditions. In addition, the most interesting materials could be used in breeding programs as parental genotypes to obtain apple cultivars with better adaptation to the limiting Spanish agro-climatic conditions.

Keywords: full bloom, fruit weight, fruit firmness, over color, fruit quality.

1. Introduction

Apple (*Malus × domestica* Borkh) is the second most important tree fruit crop of the Rosaceae family in terms of production in Spain, after peach (FAOSTAT, 2012). The Ebro basin (Northeastern Spain) is the greatest production area in Spain with 64 % of the total apple production (MAGRAMA, 2013). However, for the last three decades, apple production in Spain has decreased around 50%, from 1.037.000 t and 59.300 ha in 1985 to 481.516 t and 30.792 ha in 2012 (MAGRAMA, 2013). The altitude (around 300 m or less), the particular climatic conditions from the centre of the Ebro Valley (high temperatures and low humidity during summer, and low annual precipitation) and yet the unknown climate change effects could help to obtain poor fruit quality (low firmness, less blush and high sunburn incidence), so they do not boast a good apple production in this area. Obtaining suitable color is a problem in areas with hot summers and conditions that are not appropriate for fruit color development, as is the case in most southern European countries (Iglesias and Echeverría, 2009). Also, previous studies have reported changes in plant phenology and changes in taste and textural apple attributes in response to recent climate warming (Fujisawa and Kobayashi, 2010; Sugiura et al., 2013).

Current market demands have led Spanish growers to base their apple production on non-native cultivars, mainly from Golden, Gala, Red Delicious, Fuji, Reinette and Granny Smith groups (Casals and Iglesias, 2013), to the detriment of the locally well-adapted apple cultivars. The replacement of local by commercial apple cultivars in the last decades has led to a dramatic loss of genetic diversity in terms of adaptability, tolerance to diseases and fruit quality in Spain, as in other countries. Therefore, the recognition of the need for the collection and preservation of endangered fruit germplasm has encouraged the establishment of genetic resources conservation programs in Spain (Urrestarazu et al., 2012; Font i Forcada et al., 2014). Keulemans (1993) pointed out the interest in local cultivars for their contribution to variability and better ecological adaptability. Germplasm repositories show the development and improvement of apple and contain a vast reservoir of desirable genes (Janick et al. 1996). The first Spanish germplasm collection comprised of local and non-native apple cultivars was established in the late 1950s at the Experimental Station of Aula Dei - CSIC (EEAD-CSIC) in Zaragoza (Cambra and Ibarz, 1975). Following that initiative and more recently, different apple collections have been created in different Spanish

regions, and have all been integrated in the National Network for Plant Genetic Resources (INIA, 2013).

Genetic diversity in apple has been traditionally assessed by phenotypic and morphologic descriptors traditionally developed by the International Union for the Protection of New Varieties of Plants (UPOV, 2005) and the International Board for Plant Genetic Resources (IBPGR, 1982). Nevertheless, molecular identification using DNA markers has become the main tool for the characterization and management of germplasm collections for most fruit species (Fernández et al., 2009). Indeed, microsatellites or SSRs are the preferred DNA markers technique for the study of genetic relationship among species and the assessment of genetic diversity within crop species (Gupta et al. 1996), due to their high polymorphism level, abundance, codominant inheritance (Fernández et al., 2009), reproducibility and relative ease of analysis (Schlötterer, 2004). Many authors have used this technique to determine the genetic identities and the genetic diversity, and to identify duplicates and genetic relationships among *Malus x domestica* accessions (Guilford et al., 1997; Oraguzie et al., 2005; Gasi et al., 2010; Patzak et al., 2012) and *Malus* spp. (Hokanson et al., 1998, 2001; Guarino et al., 2006) around the world. Similarly, local apple cultivars from different Spanish regions have been also characterized by this technique (Pereira-Lorenzo et al., 2007, 2008; Díaz-Hernández et al., 2007; Ramos-Cabrer et al., 2007; Urrestarazu et al., 2012). However, no studies have reported basic and detailed information about adaptability and fruit quality traits of *Malus domestica* accessions originating from different areas of Spain and specifically when grown under the vulnerable Ebro Valley climatic conditions. Adaptability includes agronomic and morphological traits which are the basic information for breeding programs, the management of genetic resources, the protection of cultivars and the selection of candidates to diversify local production.

Therefore, the objectives of this work were (1) to determine the accession variability based on agronomic, morphological, and fruit quality traits under the Ebro basin climatic conditions, (2) to identify the main sources of variability for all analyzed accessions and the associations between traits evaluated, (3) to identify the best adapted accessions under the limiting climatic conditions of the central part of the Ebro Valley, and (4) provide useful information for breeders to select Spanish local candidates for breeding programs, or for producers aiming to diversify apple production.

2. Material and Methods

2.1. Plant Material

A total of 80 accessions [*Malus × domestica* Borkh] from the apple germplasm collection at the Experimental Station of Aula Dei (CSIC-Zaragoza, Spain) were selected (Table 1). Regarding skin fruit color, the study included 49 bicolor accessions, 22 green accessions, 8 yellow accessions and 1 brown accession, with brown corresponding to skin completely covered by russetting. These accessions, composed mostly of old local apple cultivars, were surveyed and collected in different regions of Spain under different climate influence: Atlantic (Asturias, Cantabria, Guipúzcoa, Lugo and Vizcaya); Continental (Huesca, La Rioja, Lérida, Navarra, Salamanca, Teruel, and Zaragoza); and Mediterranean (Barcelona, Gerona, Balears and Valencia).

The apple Germplasm Bank is located in the centre of the Ebro basin (Northeastern Spain, Zaragoza), on a heavy and calcareous soil, with 27% total calcium carbonate, 8% active lime, water pH 8.3, and a clay-loam texture. In this area, the climate is arid or semi-arid (Salvador et al., 2011). The average annual precipitation amounts to 345 mm, diurnal temperature variation amounts to 13.3 °C, and the reference evapotranspiration ranges from 997 mm to 1140 mm (Salvador et al., 2011). For the summer period (June-September), the average values of precipitation, diurnal temperature variation and evapotranspiration are 90 mm, 15.3 °C and 874 mm, respectively. Meteorological data were recorded by an automated station located very close to the apple Germplasm Bank. The average annual daily temperature (T_m), the average annual minimum daily temperature (T_{min}), and the average annual maximum daily temperature (T_{max}) were then calculated for the studied period. Mean value of each temperature is shown in the Electronic Supplementary Material (ESM 1).

Each accession has three-tree replications established in a unique block design. Trees were trained to a low density open-vase system (6 m × 5 m). Cultural management practices, such as fertilization and winter pruning, were conducted as in a commercial orchard. Trees were hand-thinned at 40-45 days after full bloom (DAFB) leaving one fruit per cluster. The orchard was flood irrigated every 12 days during the summer. Fruits were harvested when fruit firmness (FF) on those most exposed to sun radiation attained a value around 70-80 N and/or when they exhibited the ground color representative for each accession. All parameters were obtained over 3 to 11 years, in

the period between 2003 and 2013, and for data analysis annual average values were used.

2.2. Ploidy characterization

In the spring newly expanded mature leaves were collected from each accession evaluated. Suspensions of intact nuclei were prepared by chopping with a scalpel blade 0.5 cm² of leaf material in a plastic Petri dish containing 0.5 ml of CyStain UV ploidy, which contains 4',6-diamidino-2-phenylindole (DAPI). Nuclei suspensions were filtered through a 20 µm filter (to remove tissue debris and whole cells) and then kept at ambient temperature at least 3 min. Finally, 1 ml of filtered milli-Q water was added to nuclei suspension. Samples were analyzed on a flow cytometer (Cytometer PAS, PARTEC) equipped with a multi-parameter data acquisition and UV laser. All analyses were performed using peak-height detection (>6000 fluorescent events, e.g., nuclei, were analysed per sample) and logarithmic amplification. Data were displayed as histograms of the number of nuclei (or frequency) along the y-axis vs. the relative fluorescence intensity on the x-axis.

2.3. Phenotypic and fruit quality characterization

Blooming date was recorded for each accession according to Fleckinger (1964) method. The average date for full bloom (FB) and harvest date (HD) were scored for each accession. Type of leaf blade stipules was determined directly in the field per each accession in June. Fruits were hand-picked at commercial maturity by a single person to keep consistency of maturity grade. After that, ten typical fruits were selected from each accession, excluding terminal fruits as defined by IBPGR (1982). Mean values of FB and HD for each accession and each year is shown in the Electronic Supplementary Material (ESM 2).

Morphological and biometric features, as well as fruit quality traits were determined in the laboratory: fruit height (FH), fruit diameter in side direction (FD), length of stalk (LS), thickness of stalk (TS), width of stalk cavity (WS), depth of stalk cavity (DS), width of eye basin (WE), depth of eye basin (DE), fruit weight (FW), percentage of over color (OC), color parameters (L*, a*, b*, C*, h*), flesh firmness (FF), soluble solids content (SSC); titratable acidity (TA), hue of over color, pattern of over color and flesh color.

FH, FD, LS, TS, WS, DS, WE and DE were measured to an accuracy of 0.1 mm, using a digital calliper (Mitutoyo, Japan). Fruit shape was calculated based on FH/FD ratio. Fruit weight (FW) was determined using a digital scale (Acculab, VIC-612). Percentage of over color (OC) was visually evaluated. Color determinations were measured on the two opposite sides of the fruits (shaded and blushed). Values of L^* (brightness or lightness), a^* ($-a^*$ = greenness, $+a^*$ = redness), b^* ($-b^*$ = blueness, $+b^*$ = yellowness), C^* (chroma) and h^* (lightness's angle) were measured using a colorimeter (Chroma Meter, CR-400 Konica Minolta, Japan). FF was measured using a penetrometer (Model FT-327) on two opposite sides of each fruit after removing 1 mm thick disk of skin, with a 11 mm plunger tip. The two readings were averaged for each fruit and data were expressed in Newtons (N). SSC and TA were determined on flesh juice extracted by an automatic juicer (Philips, HR185890). SSC of fruit juice was measured with a digital refractometer (Atago PR-101, Tokyo, Japan) and was expressed as °Brix. TA was determined manually titrating 5 mL of juice with 0.1 N NaOH to a pH end point of 8.2, with 1% phenolphthalein as an indicator. Results were expressed as g malic acid per liter. Ripening index (RI) was calculated based on the SSC/TA ratio.

Qualitative traits such as hue of over color, pattern of over color and flesh color were assigned according to UPOV (2005) classification. Hue of over color was: orange red, pink red, red, purple red, and brown red. Pattern of over color was: only solid flush, solid flush with weakly defined stripes, solid flush with strongly defined stripes, weakly defined flush with strongly defined stripes, only stripes (no flush), flushed and mottled, and flushed, striped and mottled. Flesh color was: white, cream, yellowish, greenish, pinkish, and reddish.

2.4. Statistical analysis

All statistical analyses were performed using the SPSS 19.0 program (SPSS, Inc., Chicago, USA). When data was denoted through percentages of proportions, an arcsine transformation was conducted to ensure a normal distribution. To compare different leaf blade stipules (foliaceous vs. filiform) a t test ($P \leq 0.05$) was used. When ANOVA of hue of over color, patterns of over color, flesh color and fruit type, showed significant differences for climates a Duncan test at the $P \leq 0.05$ level of significance was used. Correlations between traits to reveal possible relationships were calculated using the Pearson correlation coefficient at $P \leq 0.05$. Principal component analysis (PCA) was performed using Unscrambler X 10.3 software (CamoAsa, 2001). Agronomic,

morphological and fruit quality relationships among accessions were estimated using Ward's method (Ward, 1963). A dendogram was generated using SSPS 19.0 program.

3. Results

3.1. Accession influence

The 80 accessions evaluated in this study exhibited moderate phenotypic variation for all traits evaluated (Table 2). Mean value and standard error of each trait for each accession studied is shown in Electronic supplementary material (ESM 3, ESM 4 and ESM 5).

In this study, the averages of full bloom date and harvest date were 102 Julian days and 247 Julian days respectively, but both traits showed wide range. The earliest accessions to bloom were 'Marinera' (81 Julian days) and 'Transparente' (92 Julian days) while 'Cella' (125 Julian days) was the latest. 'Marinera' (175 Julian days) and 'Bellaguardia Lardero' (178 Julian days) were the earliest accessions to be harvested, while 'Taüll-1' and 'Ortell 413 AD' were the latest ones with 295 and 296 Julian days respectively.

Biometric features of fruit, such as FH, FD, LS, TS, WS, DS, WE and DE also showed a wide variability (Table 2). FH ranged from 50.2 mm to 74.7 mm, with 'Cul de Cirio' (74.7 mm), followed by 'Perruco de Caparrosó' (74.4 mm) and 'Camuesa Fina de Aragón' (72.3 mm) showing the highest values. FD varied among accessions from 56.2 mm to 88.3 mm, with 'Audiena de Oroz' (88.1 mm) and 'Urarte' (88.3 mm) having the highest values. Length of stalk (LS) and thickness of stalk (TS) showed the minimum/maximum value ratios of 3.8 and 2.1 respectively. 'De Agosto' and 'Rebellón' showed the highest LS values (23.9 mm and 20.9 mm respectively), while 'Boluaga' and 'Audiena de Oroz' had the highest TS values. Regarding width of stalk cavity (WS), the highest values were observed in 'Camuesa de Aragón' (41.9 mm) and 'Audienza de Oroz' (39.6 mm). 'Urteberte' and 'Ortell' (3546 AD) showed the highest stalk cavity depth (DS), with 17.7 mm and 16.0 mm respectively. The highest values of eye basin width (WE) were found in the 'Audiena de Oroz' (39.5 mm) and 'Signatillis' (39.1 mm), while 'Signatillis' (10.3 mm) and 'Calvilla San Salvador' presented the highest mean for depth of eye basin (DE). Shape fruit characterized by FH/FD ratio varied from 0.73 to 1.10. 'Les-2', followed by 'Prau Riu 4' and 'Totxa' showed the lowest values, while 'Cul de Cirio', 'Del Ciri' and 'Ciri Blanc' showed the highest ones. However, 53% of the total accessions evaluated ranged from 0.75 to 0.85. A broad

range of fruit weight (FW) was observed among accessions, from 77.6 g for ‘Poma de San Juan’ to 265.8 g for ‘Audiena de Oroz’. Nevertheless, most accessions had a FW between 100 g and 200 g; four accessions had a mean FW lower than 100 g, and fourteen accessions had a mean FW higher than 200g.

Over color (OC) and the skin color characteristics are reported in Table 2. OC showed a wide range, from 0 % to 75 %. In this study, bicolor apples are the most frequent in the germplasm bank (62%), followed by green (27%), and less frequently yellow (10%) and brown (1%).

Flesh firmness (FF), soluble solids content (SSC), titratable acidity (TA) and ripening index (RI) showed a large variation among accessions. A difference of 8 °Brix was observed between accessions showing the highest (‘Terrera’, 17.8 °Brix) and the lowest SSC value (‘Calvilla San Salvador’, 10.0 °Brix). The highest maximum/minimum ratio variations were observed in TA and RI. In particular, ‘Camuesa Fina de Aragón’ had the highest TA value (17.3 g malic acid L⁻¹) and the lowest RI value (0.6), while ‘Cul de Cirio’ had the lowest TA (1.7 g malic acid L⁻¹) and the highest RI value (8.7).

3.2 Climate, pomological and genetic traits influence

Significant differences were found among accessions with different climate (Atlantic, Continental and Mediterranean), pomological traits (flesh color, leaf blade stipules, fruit type, pattern of over color and hue over color) and genetic characteristics (ploidy) (Tables 3 and 4).

Accessions with continental climate influence, with cream and white flesh, foliaceous stipules, and triploids showed the highest FD and FW values (Table 3). As usual, a strong significant positive correlation was found between FD and FW ($r = 0.90$, $P \leq 0.01$) (Table 5). LS was higher for white flesh accessions compared to cream and greenish ones, as well as for accessions with filiform stipules compared to those of the foliaceous stipules. TS and LS showed a low negative significant correlation ($r = -0.39$, $P \leq 0.01$) (Table 5). However, comparing filiform vs. foliaceous stipules this trend was not observed. White flesh, foliaceous stipules and triploid accessions showed higher WS than accessions with cream and greenish flesh and filiform stipules (Table 3). Significant positive correlations were found between WS and WE ($r = 0.79$, $P \leq 0.01$), and between these two parameters and FD ($r = 0.84$ and $r = 0.75$, $P \leq 0.01$) and FW ($r = 0.82$ and $r = 0.78$, $P \leq 0.01$) (Table 5).

With regard to FF and SSC, flesh color effect was observed in both parameters. Greenish flesh accessions presented the highest FF values, while cream flesh accessions showed the highest SSC values but the lowest TA values. No correlation was found between FF and SSC, and very low correlation was found between SSC and TA ($r = -0.19$, $P \leq 0.01$). In addition, accessions from Atlantic and Continental environments, foliaceous stipules and triploid level of ploidy showed the highest TA values (Table 3).

Fruit type, patterns of over color and hue over color had a significant effect on OC and fruit skin color parameters (L^* , a^* , b^* , C^* , h^*) in both fruit sides (shaded and blushed) (Table 4). In the case of accessions with a pattern of over color named solid flush with defined stripes and red over color they presented the highest OC values. Yellow accessions exhibited the highest L^* values in both fruit sides, while accessions with red and stripped over color showed the lowest L^* values in both sides. OC was highly and negatively correlated with L^* (blushed side) ($r = -0.71$, $P \leq 0.01$) (Table 5). Bicolor accessions and red solid flush with defined stripes showed the highest a^* values in the blushed side, and the lowest b^* , C^* and h^* values (Table 4). OC was highly and negatively correlated with h^* (blushed side) ($r = -0.76$, $P \leq 0.01$), and highly and positively correlated with a^* (red side) ($r = 0.73$, $P \leq 0.01$). In addition, a^* (blushed side) exhibited a strong negative correlation with h^* ($r = -0.78$, $P \leq 0.01$) (Table 5).

3.3. Classification

Principal component analysis was used to identify the most significant variables in the data set. From the 27 initial traits, the ratio FH/FD, C^* and h^* color parameters (from both fruits sides), and RI were not included in PCA, since they were a combination of individual traits. The first eight principal components (PCs) accounted for 85% of the variance (Table 6). Main sources of variability with the highest Eigen vectors in each PC, were as follows. PC1: fruit diameter in side direction (FD), fruit weight (FW), width and depth of eye basin (WE and DE), and stalk cavity (WS); PC2: percentage of over color (OC), b^* (shaded side), and L^* , a^* and b^* from blushed side; PC3: harvest date (HD), soluble solids content (SSC), titratable acidity (TA), and stalk length (LS); and PC4: fruit height (FH); PC5: L^* (shaded side) and depth of stalk cavity (DS); PC6: a^* (shaded side) and stalk thickness (TS); PC7: flesh firmness (FF); and PC8: full bloom date (FB).

Taking into account the variables used in the PCA analysis, a hierarchical agglomerative cluster analysis was used to divide the available data up into groups of increasing dissimilarity. The Ward's minimum variance criterion was applied. The dendrogram (Fig. 1) pointed out that, all 80 accessions were different from each other, and six clusters are identified at high level of similarity (ESS lower than 6). This division did not correspond to apple accessions grouped according to origin, fruit type, type of stipules and ploidy level. In each cluster, origin, fruit type, and type of stipules was quite diverse. The first group, which includes 'Pera de Sangüesa' through 'Boluaga' (Fig. 1), is characterized by accessions with low or none over color, medium-to-high SSC and b^* (both shaded and blushed fruit sides) values, and medium-to-long DS. The 5 bicolor accessions ('Almenar-2', 'Boluaga', 'Bossost-5', 'Les-2', and 'Verde Doncella') included in this cluster had all of them OC values lower than 30%. The accessions included in cluster 2, 'Marquinez' through 'Audiena de Oroz', showed medium-to-large FH, FD, FW, WE, DE, DS and WS values (Fig. 1). Their origin was quite diverse and most of them were bicolor. The third cluster, 'Les-1' through 'Sandía' (Fig. 1), included also accessions from different regions. Most of these accessions were bicolor, had TA values over 10 g malic acid L⁻¹, except 'Les-1' and 'Sant Joan', and had fruits with medium-to-long LS. The fourth cluster grouped 10 accessions, 'Reneta' through 'Torrera' (Fig. 1). These accessions have different origins (Alicante, Asturias, Baleares, Cantabria, Gerona, Huesca, La Rioja and Lérida). They showed a late period of flowering and ripening, high OC and a^* (blushed side), and low L^* and b^* values from both fruit sides. The fifth cluster grouped only 6 accessions, 'Camosa' through 'Eugenia' (Fig. 1). It consisted of late flowering accessions with high L^* (shaded side) and LS values. Except 'Camuesa del Llobregat', which had green skin color, the rest of the accessions were bicolor type. The final group clustered away from the other groups, and contains a large number of cultivars, from 'Mañana' to 'Esperiega'. It consisted mainly of late flowering and harvested accessions with high FF, and L^* and b^* values from both fruit sides (Fig. 1).

Comparisons of the clusters obtained by agronomic, morphological and fruit quality traits, suggest possible synonymies in the Germplasm Bank. For example, in cluster 1 both accessions named 'White Reinette of Canada' (308 AD and 3194 AD), in cluster 3 'Transparente' and 'Transparente Blanca', and 'Ascara 1' and 'Ascara 2', as well as accessions named 'Ortell' (413 AD and 3546 AD) in cluster 6, among others.

4. Discussion

Significant phenotypic diversity has been detected in the apple germplasm grown at the Experimental Station de Aula Dei (CSIC), but surveyed and collected in different regions of Spain, where apple has been grown for centuries. Under our growing conditions, most accessions (75%) concentrated their flowering between 7-20 April, and approximately 60% of the accessions evaluated were harvested between September and October. As expected, blooming and ripening time were low significantly correlated. In agreement with Mounzer et al. (2008), blooming and harvesting times may change between years, depending on the environmental conditions, especially temperature, making them particularly vulnerable to climate change. However, Tancred et al. (1995) determined that the heritability of ripening dates in *Malus x domestica* is high. In particular, earliness is a polygenic trait with high heritability and also additive genetic variance (Brown, 1960), and it is a target in various breeding programs (Tancred et al., 1995). In our germplasm collection, a total of 40% accessions were harvested earlier (before September). These could provide a local alternative to early-harvested cultivars, if further agronomic evaluation confirms their good eating quality (Ramos-Cabrer et al., 2007).

The values of FH, FD and FW varied among accessions. In this study, as reported by Ramos-Cabrer et al. (2007), FD was highly correlated with FW. Indeed, fruit size (FD and FW) is used to classify apples for fresh-market. In fact, commercial categories for apple fruit marketing are primarily based upon fruit diameter or fruit weight (RCE no. 1619/201). Some accessions, e.g. 'Audiena de Oroz' and the triploide 'Urate' have grown fairly large fruits (around 88 mm) with high weights (more than 260 g), while 'Eugenia', 'Poma de San Juan', Reneta', and 'Roja Valle Benejama' had FW lower than 100 g with FD around 65 mm or less. The average fruit weight of cultivars with fruit diameter greater than 70 mm was approx. 182 g. This could be explained by the fact that the size of the fruit was probably one of the first parameters to be considered in the traditional farmers' selection process. Oraguzie et al. (2001) estimated narrow-sense heritability for fruit weight (0.27-0.90). In addition, the major factors determining ultimate size are the cultivar, genotype and crop load. Exposure to light also plays an important part in determining final size (Dennis, 2003). In this study, accessions were grown under the same environmental conditions and cultural practices, so it could be concluded that accessions with high FW had good genetic potential for this character.

Fruit shape (FH/FD ratio) greatly varied among the studied accessions. It depends on stage of fruit development (Dennis, 2003) and on weather conditions (Jackson, 2003). It ranges from very small to very large (UPOV, 2005), and it is known to be quantitatively inherited (Brown 1960). At commercial maturity, around 9% of the total accessions evaluated showed a FH/FD ratio above 1, and they could be classified as elongated accessions, and around 8% showed a FH/FD ratio lower than 0.75, and they could be classified as flat. Therefore, most accessions evaluated in this study had a ratio considered to be medium to long.

Biometric features of stalk, stalk cavity and eye basin greatly varied among accessions, and values of LS, TS, WS and DS were within the range reported by other apple accessions studies (Santesteban et al., 2009; Gasi et al., 2011). The skin color of fruit is a very important quality attribute, together with FF, SSC and TA. Color has a significant impact on consumer perception of fruit quality, especially as regard the attractiveness of fruit (Ruiz and Egea, 2008), and it is also an important trait to classify apples for the fresh market. Pre-harvest and immediately post-harvest red color development are controlled by genotype, carbohydrate supply, direct effects of light on anthocyanin formation, temperature and nutritional factors (Jackson, 2003). In this study, 62% of the accessions evaluated were bicolor, showing few accessions more than 50% of over color, as ‘Aciprés’, ‘Cabdellá-2’, ‘Roja del Valle de Benezama’, ‘Prau Riu 3’, ‘Reneta’, ‘Nesple’ and ‘San Miguel’. However, these accessions usually were grown in areas with more altitude and latitude than the Ebro basin area, where differences between diurnal and nocturnal temperatures are higher. Consequently, they could have higher percentage of over color. Indeed, Jackson (2003) reported that a series of a few nights with temperatures in the range 2-5 °C followed by warm sunny days promotes red color development.

Fruit firmness (FF) is strongly influenced by maturity with FF decreasing as the fruit ripens (Kingston, 1991). The accessions were harvested at commercial maturity, showing a range of 47.5-112.4 N for flesh firmness (FF). This trait is relevant for the assessment of the quality of the fruit, affecting fruit shelf life, and for consumer acceptance. Harker et al. (2008) reported greater acceptability with higher firmness until a threshold value of 62 N, after which acceptance levelled off. An apple consumption study by Konopacka et al. (2010) reported that fruit consumption habit is significantly changing with age, with a tendency of higher fruit consumption by older people.

Usually, these people tend to consume apples with low firmness. Therefore, accessions with low FF and good eating quality could be better considered for marketing to ancient people. ‘Bellaguardia de Lardero’, ‘Landetxo’ and ‘Marinera’ showed the lowest FF values, but their high TA values made them not suitable to older people, which in general prefer sweeter fruits apples. FF is also strongly influenced by fruit size (Ebel et al., 1993) with smaller fruits being generally firmer than larger fruits due to a higher cellular density in the former. Nevertheless, FF was poorly correlated with FD in this study.

SSC showed a wide range in the present work: 8 °Brix difference between the minimum and maximum values, but they were within the values reported by Ramos-Cabrer et al. (2007) and Mratinić and Akšić (2012). To optimize consumer satisfaction, Echeverría et al. (2002) recommended SSC values between 13.5 and 15.5 °Brix. In this study, 10% of the total accessions showed SSC values higher than 15.5 °Brix (e.g. ‘Pera 2’, ‘Eugenia’, ‘White Reinette of Canada’, ‘Pero Pardo’, ‘Reineta Regil’, ‘Cirio’ and ‘Torrera’), 30% between 13.5° and 15.5 ° Brix, and 60% lower than 13.5 °Brix. The values of TA and SSC/TA showed the highest maximum/minimum values due to the high TA variability observed in all the accessions evaluated, and were within the range reported by Ramos-Cabrer et al. (2007). Pereira-Lorenzo et al. (2009) considered sweet apples when malic acid was lower than 4.5 g L⁻¹. In this study, only 25% of the total accessions evaluated had TA lower than that value. Some of them could be recommendable for fresh market because they are firm, sweet and have medium to low acidity, such as ‘Del Ciri’, ‘Cul de Cirio’, ‘San Miguel’, ‘Cella’, ‘Almenar-2’ and ‘Ruixou-1’. On the contrary, most of the accessions from this study could be useful for cider production (Ramos-Cabrer et al., 2007).

Concerning the influence of climate, pomological and genetic traits on agronomic, morphological and fruit quality traits, different results were obtained. In general, the highest FD and FW values were observed for accessions with continental climate influence, cream and white flesh, foliaceous stipules, and triploids. Ramos-Cabrer et al. (2007) also reported that triploid accessions produced heavier fruits than diploids. The significant positive correlation between FD and FW agrees with previous reports in apple (Janick et al., 1996; Gasi et al., 2010; Ramos-Cabrer et al., 2007), as well as in cherry studies (Petrucelli et al., 2013). On the other hand, Isuzugawa et al. (2014) reported that the increase of chromosome number results in the expansion of cells,

leading to increase fruit size. In this study no correlation was observed between ploidy level and FD, although in general triploid accessions had higher FD than diploid accessions. A study on transgenic tomato plants (Centeno et al., 2011) suggested that acid malic is important for the enlargement of fruits. In this study, triploids accessions showed larger FD and higher TA than diploid accessions, although ploidy level was not correlated with FD and TA (Table 5), underlying that TA role is not clear in the control of apple fruit size.

Regarding fruit type, pattern of the over color and hue over color, a great influence on fruit color (OC, L*, a*, b*, C*, h*) parameters was reported. In agreement with some studies in apple (Iglesias and Echeverría, 2009; Iglesias et al., 2012), the accessions with higher coloring included high average surface color and tended to show low hue values, such as ‘Reneta’, ‘Nesple’ and ‘San Miguel’. Moreover, other authors also reported that higher average of red fruit color surface and low hue angle were associated with high anthocyanin content in ‘Fuji’ strains (Veberic et al., 2007) and ‘Gala’ strains (Sturm et al., 2003; Iglesias and Echeverría, 2009). Although apples tend to show lower anthocyanin content than other fruits, the variability observed in this germplasm collection could be useful to improve attractiveness and antioxidants content in new apple cultivars. Indeed, the consumption of high levels of antioxidants is promoted as being beneficial to one’s long-term health by reducing general oxidative stress.

Multivariate analysis has been used to study different germplasm collections of *Prunus* spp. (Pérez et al., 1993; Font i Forcada et al., 2014) and *Malus × domestica* (Gasi et al., 2011; Pereira-Lorenzo et al., 2003). In this study, PCA analysis showed that all variables studied had high scores in the first eight principal components. The traits that revealed to be main sources of variability in the first principal component corresponded to FW, FD, TS, WE, DE and WS. These results agree with Pereira-Lorenzo et al. (2003) studying different local apple cultivars grown in Northwestern of Spain, as well as with Gasi et al. (2011) working with autochthonous and foreign apple cultivars grown in Bosnia-Herzegovina.

The cluster analysis of the 80 accessions produced several groupings based mainly on agronomic, morphological, and fruit quality traits. There was not a straightforward correspondence between origin and their placement in the dendrogram, which agrees to the traditional exchanges of plant material through grafting, mainly between different areas in the Ebro basin (Urrestarazu et al., 2012). The results also revealed the existence

455 of variability in the Germplasm Bank maintained at the EEAD. Nevertheless, some
 456 possible synonymies were found and could be repeats in the Germplasm Bank, e.g.
 457 ‘White Reinette of Canada’ (308 EEAD and 3194 EEAD) and ‘Ortell’ (413 AD and
 458 3546 AD). These accessions had similar phenotypic characteristics and the same allelic
 459 SSR profile (Urrestarazu et al., 2012). These synonymies were expected, as the
 460 accessions received very similar or identical denominations. ‘Ortell’ is considered an
 461 old Spanish cultivar and ‘White Reinette of Canada’ is an international cultivar
 462 introduced in Spain long time ago. Reinettes are a group of cultivars difficult to define
 463 since it includes yellow skin apples such as ‘White Reinette’, bicolor type such as
 464 ‘Reineta Encarnada’ and brown type such as ‘Reineta Gris’ (Pereira-Lorenzo et al.,
 465 2007), but in general they have obloid fruits with sweet flesh and little and medium
 466 acidity. Four accessions named Reineta were included in the present study, two
 467 clustered together as mentioned above, and the other two clustered separately. ‘Reineta
 468 Inesita Asua’ grouped closer to ‘Marquinez’, ‘Navalmoral de Bejar-1’ and ‘Bossot-1’ in
 469 cluster 2, although Urrestarazu et al. (2012) reported the same allelic profile for ‘White
 470 Reinette of Canada (308 AD and 3194 AD) and ‘Reineta Inesita Asua’. This suggests
 471 some clonal mutation or strain variation confirmed with morphology, but not detected
 472 with SSRs. In fact, one accession produces green apples and the other bicolor, and both
 473 clustered differently in the present study. In addition, Pereira-Lorenzo et al. (2007)
 474 reported that ‘Esperiega’ was an introgression of Reinette group in local populations.
 475 This study only can corroborate that ‘Esperiega’ and ‘Reineta Regil’ had similar
 476 phenotypic profile. On the contrary, ‘Reineta Regil’ grouped closer to ‘Valsaina’ in
 477 cluster 6, but according to Urrestarazu et al. (2012) they had different allelic profile.
 478 Similar result was observed between ‘Ascara 1’ and ‘Ascara 2’, and ‘Transparente
 479 Blanca’ and ‘Transparente’. They grouped closely in the dendogram, but they do not
 480 share the same allelic profile (Urrestarazu et al., 2012). On the other hand, some
 481 accessions with the same ploidy level, and the same or different name and geographical
 482 origin, which grouped closely in the dendogram, could be possible synonymies. That
 483 was the case of ‘Pera de Sangüesa’ and ‘Pero Pardo’, and ‘De Pera’ and ‘Pera 2’,
 484 ‘Bossost-5’, ‘Les-2’ and ‘Prau Riu 4’ from cluster 1. ‘Astrakan Roja’ and ‘Landetxo’
 485 from cluster 3, ‘Reneta’ and ‘Roja Valle Benejama’ from cluster 4, and ‘Mañaga’ (3554
 486 AD), ‘Reguard-1’ and ‘Irgo-2’, and ‘Ciri Blanc’ and ‘Del Ciri’ from cluster 6.

As a whole, eight groups of synonymies have been found which represents 21% of the total accessions studied. All these synonymies named differently had equal genetic and phenotypic profile based on the variables evaluated on cluster analysis. However, as mentioned above, mutations could not be detected with SSR. This is the case, for example, of ‘Astrakan Roja’ and ‘Landetxo’. They share the same allelic profile but they show phenotypic differences, such as pattern of over color and fruit ribbing (Fig. 2). On the other hand, duplication within a collection must be avoided due to the high maintenance cost of field collections (Hokanson et al., 1998), but a certain level of duplication provides a safety backup system (Urrestarazu et al., 2012).

5. Conclusions

Agronomic, morphological and fruit quality data using principal component analysis and cluster analysis revealed the existence of a wide variability in the apple Germplasm Bank maintained at the Experimental Station de Aula Dei (CSIC). The knowledge of the phenotypic diversity among accessions is a key point for the efficient use and conservation of traditional material with high risk of extinction. Furthermore, this information could be used for protection or patenting processes of new apple cultivars carried out by EU-Community Plant Variety Office.

From the eight groups of synonymous found in this study, several accessions with the same allelic profile exhibit phenotypic differences. This suggests the existence of mutations not detected with molecular markers, showing the interest of phenotypic characterization and biometric studies to manage germplasm collections. Precise phenotypic characterization in conjunction with genetic analysis could result in a better understanding of the real germplasm diversity available.

This study provides useful information of agronomic, morphological and fruit quality traits of accessions grown under the Ebro Valley vulnerable climatic conditions. In addition, those interesting materials can be used in future breeding programs as parental genotypes searching for future agronomic, morphological and fruit quality improvement through genetic recombination, as well as to plan a strategy to bred apple cultivars with better adaptation to the limiting Spanish agro-climatic conditions.

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523 **6. References**

524 Brown, A.G., 1960. The inheritance of shape, size and season of ripening in progenies
525 of the cultivated apple. *Euphytica* 9, 327–337.

526 Cambra, R., Ibarz, P., 1975. Variedades de manzano en España. *An. Estac. Exp. Aula*
527 *Dei* 13 (1/2), 1–97.

528 CamoASA, 2001. The unscrambler 9.6 user manual. CamoASA, Oslo.

529 Casals, E., Iglesias, I., 2013. Situación actual de la producción de manzana en España y
530 análisis de la campaña actual. *Vida Rural* 357, 27–35.

531 Centeno, D.C., Osorio, S., Nunes-Nesi, A., Bertolo, A.L., Carneiro, R.T., Araújo, W.L.,
532 Steinhauser, M.C., Michalska, J., Rohrmann, J., Geigenberger, P., Oliver, S.N., Stitt,
533 M., Carrari, F., Rose, J.K., Fernie, A.R., 2011. Malate plays a crucial role in starch
534 metabolism, ripening, and soluble solid content of tomato fruit and affects postharvest
535 softening. *Plant Cell* 23, 162–184.

536 Dennis, F., 2003. Flowering, pollination and fruit set and development, in: Ferree, D.C.,
537 Warrington, I.J. (Eds.), *Apple, Botany, Production and Uses*. CAB International,
538 Wallingford, pp. 153–166.

539 Díaz-Hernández, M.B., Ramos-Cabrer, A.M., Pereira-Lorenzo, S., 2007. Estudio
540 comparativo de los principales cultivares de manzano (*Malus x domestica* Borkh.) de
541 Asturias, País Vasco y Galicia. INIA, Madrid.

542 Ebel, R.C., Proebsting, E.L., Patterson, M.E., 1993. Regulated deficit irrigation may
543 alter apple maturity, quality and storage life. *HortScience* 28, 141–143

544 Echeverría, G., Graell, J., López, M.L., 2002. Effect of harvest date and storage
545 conditions on quality and aroma production of ‘Fuji’ apples. *Food Sci. Technol. Int.* 8,
546 351–360.

547 FAOSTAT, 2012. (<http://www.faostat.fao.org/>)

548 Fernández, A., Alonso, J.M., Espiau, M.T., Rubio-Cabetas, M.J., Socias, R., 2009.
 549 Genetic diversity in Spanish and Foreign Almond Germplasm assessed by molecular
 550 characterization with simple sequence repeats. J. Amer. Soc. Hortic. Sci. 134 (5), 535–
 551 542.

552 Fleckinger, J., 1964. Phénologie et arboriculture fruitière. In: Grisvard, P., Chaudun,
 553 V.C. (Eds.), Le bon jardinier (Tome I, 2ème partie). La Maison Rustique, Paris, pp.
 554 362–372.

555 Font i Forcada, C., Gradziel, T.M., Gogorcena, Y., Moreno, M.A., 2014. Phenotypic
 556 diversity among local Spanish and foreign peach and nectarine [*Prunus persica* (L.)
 557 Batsch] accessions. Euphytica 197, 261–277.

558 Fujisawa, M., Kobayashi, K., 2010. Apple (*Malus pumila* var. *domestica*) phenology is
 559 advancing due to rising air temperature in northern Japan. Global Chang. Biol. 16,
 560 2651–2660.

561 Gasi, F., Simon, S., Pojskic, N., Kurtovic, M., Pejic, I., 2010. Genetic assessment of
 562 apple germplasm in Bosnia and Herzegovina using microsatellite and morphologic
 563 markers. Sci. Hortic. 126, 164–171.

564 Gasi, F., Simon, S., Pojskic, N., Kurtovic, M., Pejic, I., 2011. Analysis of morphological
 565 variability in Bosnia and Herzegovina's autochthonous apple germplasm. J. Food Agric.
 566 Environ. 9, 444–448.

567 Guarino, C., Santoro, S., De Simona, L., Lain, O., Cipriano, G., Testolin, R., 2006.
 568 Genetic diversity in a collection of ancient cultivars of apple (*Malus × domestica*
 569 Borkh.) as revealed by SSR-based fingerprinting. J. Hortic. Sci. Biotechnol. 81, 39–44.

570 Guilford, P., Prakash, S., Zhu, J.M., Rikkerink, E., Gradiner, S., Basset, H., Forster, R.,
 571 1997. Microsatellites in *Malus x domestica* (apple): abundance, polymorphism and
 572 cultivar identification. Theor. Appl. Genet. 94, 249–254.

573 Gupta, P.K., Balyan, H.S., Sharma, P.C., Ramesh, B., 1996. Microsatellites in plants: A
 574 new class of molecular markers. Curr. Sci. 70, 45–54.

575 Harker, F.R., Kupferman, E.M., Marin, A.B., Gunson, F.A., Triggs, C.M., 2008. Eating
 576 quality standards for apples based on consumer preferences. Postharvest Biol. Technol.
 577 50, 70–78.

578 Hokanson, S.C., Szewc-Mcfadden, A.K., Lamboy, W.F., McFerson, J.R., 1998.
 579 Microsatellite (SSR) markers reveal genetic identities, genetic diversity and
 580 relationships in a *Malus x domestica* borkh. core subset collection. Theor. Appl. Genet.
 581 97, 671–683.

582 Hokanson, S.C., Lamboy, W.F., Szewc-Mcfadden, A.K., McFerson, J.R., 2001.
 583 Microsatellite (SSR) variation in a collection of *Malus* (apple) species and hybrids.
 584 Euphytica 118, 281–294.

585 IBPGR, 1982. Descriptor list for apple (*Malus*). International Board Plant Genetic
 586 Resources, Rome.

587 Iglesias, I., Echeverría, G., 2009. Does strain affect fruit color development,
 588 anthocyanin content and fruit quality in ‘Gala’ apples? A comparative study over three
 589 seasons. J. Am. Pomol. Soc. 63, 168–180.

590 Iglesias, I., Echeverría, G., López, L., 2012. Fruit color development, anthocyanin
 591 content, standard quality, volatile compound emissions and consumer acceptability of
 592 several ‘Fuji’ apple strains. Sci. Hortic. 137, 138–147.

593 INIA, 2013. (<http://www.inia.es/>)

594 Isuzugawa, K., Murayama, H., Nishio, T., 2014. Characterization of a giant-fruit mutant
 595 exhibiting fruit-limited polyploidization in pear (*Pyrus communis* L.). Sci. Hortic. 170,
 596 196–202.

597 Jackson, J.E., 2003. The Biology of Apples and Pears. The Biology of Horticultural
 598 Crops. Cambridge, Cambridge University Press.

599 Janick, J., Cummins, J.N., Brown, S.K., Hemmat, M., 1996. Apples. In: Janick, J.,
 600 Moore, J.N. (Eds.), Fruit Breeding, Tree and Tropical fruits. Wiley, New York, pp. 1–
 601 77.

602 Keulemans, J., 1993. Perspectives nouvelles en amélioration du pommier. Le Fruit
 603 Belge, 445, 147–151.

604 Kingston, C.M., 1991. Maturity indices for apple and pear. Hortic. Rev. 13, 407–432.

605 Konopacka, D., Jesionkowska, K., Kruczynska, D., Stehr, R., Schoorl, F., Buehler, A.,
 606 Egger, S., Codarin, S., Hilaire, C., Hofler, I., Guerra, W., Liverani, A., Donati, F.,
 607 Sansavini, S., Martinelli, A., Petiot, C., Carbo, J., Echeverría, G., Iglesias, I., Bonany,

608 J., 2010. Apple and peach consumption habits across European countries. *Appetite* 55,
609 478–483.

610 MAGRAMA, 2013. (<http://www.magrama.gob.es/>)

611 Mounzer, O.H., Conejero, W., Nicolás, E., Abrisqueta, I., García-Orellana, Y.V., Tapia,
612 L.M., Vera, J., Abrisqueta, J.M., Ruíz-Sánchez, M.C., 2008. Growth pattern and
613 phenological stages of early-maturing peach trees under a Mediterranean climate.
614 *HortScience* 43, 1813–1818.

615 Mratinić, E., Akšić, M.F., 2012. Phenotypic diversity of apple (*Malus* sp.) germplasm in
616 South Serbia. *Braz. Arch. Biol. Technol.* 55 (3), 349–358.

617 Oraguzie, N.C., Hofstee, M.E., Brewer, L.R., Howard, C., 2001. Estimation of genetic
618 parameters in a recurrent selection program in apple. *Euphytica* 118, 29–37.

619 Oraguzie, N.C., Yamamoto, T., Soejima, J., Suzuki, T., De Silva, H.N., 2005. DNA
620 fingerprinting of apple (*Malus* spp.) rootstocks using simple sequence repeats. *Plant*
621 *Breed.* 124, 197–202.

622 Patzak, J., Paprštein, F., Henychová1, A., Sedlák, J., 2012. Genetic diversity of Czech
623 apple cultivars inferred from microsatellite markers analysis. *Hortic. Sci.* 39(4), 149–
624 157.

625 Pereira-Lorenzo, S., Ramos-Cabrer, A.M., Ascasibar-Errasti, J., Piñeiro-Andión, J.,
626 2003. Analysis of apple germplasm in Northwestern Spain. *J. Am. Soc. Hortic. Sci.*
627 128(1), 67–84.

628 Pereira-Lorenzo, S., Ramos-Cabrer, A.M., Díaz-Hernández, M.B., 2007. Evaluation of
629 genetic identity and variation of local apple cultivars (*Malus x domestica* Borkh.) from
630 Spain using microsatellite markers. *Genet. Resour. Crop. Evol.* 54, 405–420.

631 Pereira-Lorenzo, S., Ramos-Cabrer, A.M., Gonzalez-Díaz, A.J., Díaz-Hernández, M.B.,
632 2008. Genetic assessment of local apple cultivars from La Palma, Spain, using simple
633 sequence repeats (SSRs). *Sci. Hortic.* 117, 160–166.

634 Pereira-Lorenzo, S., Ramos-Cabrer, A.M., Fischer, M. 2009. Breeding apple (*Malus x*
635 *domestica* Borkh), in: Jain, M.S., Priyadarshan, P.M. (Eds.), *Breeding Plantation Tree*
636 *Crops, Temperate Species*. Springer Science+Business Media, New York, pp. 33–82.

637 Pérez, S., Montes, S., Mejía, C. 1993. Analysis of peach germplasm in Mexico. J.
638 Amer. Soc. Hortic. Sci. 118, 519–524.

639 Petruccelli, R., Ganino, T., Ciaccheri, L., Maselli, F., Mariotti, P., 2013. Phenotypic
640 diversity of traditional cherry accessions present in the Tuscan region. Sci. Hortic. 150,
641 334–347.

642 Ramos-Cabrer, A.M., Díaz-Hernández, M.B., Pereira-Lorenzo, S. 2007. Morphology
643 and microsatellites in Spanish apple collections. J. Hortic. Sci. Biotechnol. 82(2), 257–
644 265.

645 Ruiz, D., Egea, J., 2008. Phenotypic diversity and relationships of fruit quality traits in
646 apricot (*Prunus armeniaca* L.) germplasm. Euphytica 163, 143–148.

647 Salvador, R., Martínez-Cob, A., Caverro, J., Playán, E., 2011. Seasonal on-farm
648 irrigation performance in the Ebro basin (Spain): crops and irrigation systems. Agric.
649 Water Manag. 98, 577–587.

650 Santesteban, L.G., Miranda, C., Royo, J.B., 2009 Assessment of the genetic and
651 phenotypic diversity maintained in apple core collections using either agro-morphologic
652 or molecular marker data. Spanish J. Agric. Res. 7(3), 572–584.

653 Schlötterer, C., 2004. The evolution of molecular markers - just a matter of fashion?
654 Nat. Rev. Genet. 5, 63–69.

655 Sugiura, T., Ogawa, H., Fukuda, N., Moriguchi, T., 2013. Changes in the taste and
656 textural attributes of apple in response to climate change. Sci. Rep. 3, 1–7.

657 Sturm, K., Hudina, M., Solar, A., Marn, M.V., Stampar, F., 2003. Fruit quality of
658 different ‘Gala’ clones. Eur. J. Hortic. Sci. 68, 169–175.

659 Tancred, S.J., Zeppa, A.G., Cooper, M., Stringer, J.K., 1995. Heritability and patterns of
660 inheritance of the ripening date of apples. HortScience 30, 325–328.

661 Urrestarazu, J., Miranda, C., Santesteban, L.G., Royo, J.B., 2012. Genetic diversity and
662 structure of local apple cultivars from Northeastern Spain assessed by microsatellite
663 markers. Tree Genet. Genomes 8, 1163–1180.

664 UPOV, 2005. International Union for the protection of new varieties of plants.
665 Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability to Apple
666 (*Malus x Domestica* Borkh.). TG/14/9.

- 667 Veberic, R., Zadavec, P., Stampar, F., 2007. Fruit quality of 'Fuji' apple (*Malus*
668 *domestica* Borkh.) strains. J. Sci. Food Agric. 87, 593–599.
- 669 Ward, J., 1963. Hierarchical grouping to optimize an objective function. J. Am. Stat.
670 Assoc. 58, 236–244.

Table 1 Characteristics of the 80 apple accessions (*Malus x domestica*) evaluated and established in the germplasm collection at the Experimental Station of Aula Dei – CSIC

Accessions	Accession number	Origin	Fruit type	Flesh colour	Hue over color	Patern over colour	Leaf blade stipules	Ploidy
Aciprés	3339 AD	Huesca, SP	Bicolor	Cream	Red	Only solid flush	Foliaceous	2
Almenar-2	3555 AD	Lérida, SP	Bicolor	Cream	Orange red	Solid flush with defined stripes	Foliaceous	2
Ascara 1	3423 AD	Huesca, SP	Bicolor	Cream	Red	Only stripes	Foliaceous	3
Ascara 2	3424 AD	Huesca, SP	Bicolor	Yellowish	Red	Only stripes	Filiform	2
Astrakán Roja	3378 AD	Navarra, SP	Bicolor	White	Red	Solid flush with defined stripes	Filiform	2
Audiena de Oroz	3375 AD	Navarra, SP	Green	White	-	-	Foliaceous	2
Bellaguardia Lardero	3547 AD	La Rioja, SP	Yellow	White	Orange red	Only solid flush	Filiform	2
Boluaga	3340 AD	Guipúzcoa, SP	Bicolor	Yellowish	Red	Only stripes	Foliaceous	3
Bossost-1	3626 AD	Lérida, SP	Bicolor	Greenish	Orange red	Solid flush with defined stripes	Foliaceous	3
Bossost-4	3629 AD	Lérida, SP	Bicolor	Greenish	Pink red	Only solid flush	Filiform	2
Bossost-5	3630 AD	Lérida, SP	Bicolor	Cream	Pink red	Solid flush with defined stripes	Foliaceous	3
Bost Kantoia	3341 AD	Guipúzcoa, SP	Yellow	Cream	-	-	Filiform	2
Cabdellá-2	3613 AD	Lérida, SP	Bicolor	Cream	Red	Only solid flush	Foliaceous	2
Calvilla de San Salvador	3342 AD	Zaragoza, SP	Bicolor	Greenish	Red	Only solid flush	Foliaceous	2
Camosa	3620 AD	Lérida, SP	Bicolor	Cream	Orange red	Only stripes	Filiform	2
Camuesa del Llobregat	1342 AD	Barcelona, SP	Green	White	-	-	Filiform	2
Camuesa fina de Aragón	3372 AD	Huesca, SP	Bicolor	White	Purple red	Flushed and mottled	Foliaceous	2
Cella	2512 AD	Teruel, SP	Green	Cream	-	-	Filiform	2
Ciri Blanc	3402 AD	Gerona, SP	Green	White	-	-	Filiform	2
Cirio	3615 AD	Lérida, SP	Green	Cream	-	-	Filiform	2
Cul de Cirio	3551 AD	Lérida, SP	Bicolor	Cream	Orange red	Only solid flush	Filiform	2
De Agosto	3619 AD	Lérida, SP	Bicolor	Cream	Pink red	Only stripes	Filiform	2
De pera	3416 AD	La Rioja, SP	Yellow	Cream	-	-	Foliaceous	2
Del ciri	3413 AD	Balears, SP	Yellow	White	-	-	Filiform	2
Esperiega	3420 AD	La Rioja, SP	Yellow	Cream	-	-	Foliaceous	2
Eugenia	3468 AD	Gerona, SP	Bicolor	Greenish	Red	Only solid flush	Foliaceous	2
Guillemes	3411 AD	Balears, SP	Bicolor	White	Red	Solid flush with defined stripes	Foliaceous	2
Helada	3368 AD	Balears, SP	Green	White	-	-	Filiform	2
Hierro	3374 AD	Navarra, SP	Bicolor	White	Red	Only solid flush	Filiform	2
Irgo-2	3622 AD	Lérida, SP	Bicolor	Cream	Pink red	Only stripes	Foliaceous	2
Landetxo	3343 AD	Navarra, SP	Bicolor	White	Red	Flushed and mottled	Filiform	2
Les-1	3624 AD	Lérida, SP	Bicolor	Cream	Orange red	Only solid flush	Foliaceous	2
Les-2	3625 AD	Lérida, SP	Bicolor	White	Red	Only stripes	Foliaceous	3
Mañaga	3554 AD	Lérida, SP	Bicolor	Cream	Pink red	Only solid flush	Filiform	2
Mañaga	469 AD	Huesca, SP	Green	Greenish	-	-	Foliaceous	2
Marinera	3412 AD	Balears, SP	Bicolor	White	Red	Only solid flush	Filiform	2
Marquinez	3419 AD	La Rioja, SP	Bicolor	White	Red	Flushed and mottled	Foliaceous	3
Montcada-1	3631 AD	Lérida, SP	Bicolor	Cream	Red	Solid flush with defined stripes	Foliaceous	2
Morro de Liebre	3256 AD	Zaragoza, SP	Bicolor	White	Red	Only solid flush	Filiform	2

Table 1 Continued

Accessions	Accession number	Origin	Fruit type	Flesh colour	Hue over color	Patern over colour	Leaf blade stipules	Ploidy
Navalmoral de Bejar-1	3548 AD	Salamanca, SP	Bicolor	Greenish	Red	Only stripes	Foliaceous	2
Nesple	3410 AD	Baleares, SP	Bicolor	Cream	Red	Solid flush with defined stripes	Foliaceous	2
Normanda	3252 AD	Zaragoza, SP	Bicolor	White	Pink red	Solid flush with defined stripes	Filiform	3
Ortell	413 AD	Zaragoza, SP	Bicolor	White	Red	Flushed and mottled	Foliaceous	3
Ortell	3546 AD	La Rioja, SP	Bicolor	White	Pink red	Flushed and mottled	Foliaceous	2
Pera 2	3417 AD	La Rioja, SP	Yellow	Cream	-	-	Foliaceous	2
Pera de Sangüesa	3379 AD	Navarra, SP	Green	Cream	-	-	Foliaceous	3
Pero pardo	3369 AD	Navarra, SP	Green	Cream	-	-	Filiform	3
Peromingán	1158 AD	Asturias, SP	Green	Greenish	-	-	Filiform	2
Peruco de Caparroso	3373 AD	Navarra, SP	Bicolor	White	Red	Only stripes	Filiform	2
Poma de San Juan	3556 AD	Lérida, SP	Bicolor	White	Red	Solid flush with defined stripes	Filiform	2
Prau Riu 3	3491 AD	Asturias, SP	Bicolor	Greenish	Red	Solid flush with defined stripes	Filiform	2
Prau Riu 4	3492 AD	Asturias, SP	Bicolor	White	Red	Solid flush with defined stripes	Foliaceous	3
Prau Riu 5	3493 AD	Asturias, SP	Green	Greenish	-	-	Foliaceous	2
Rebellón	3370 AD	Navarra, SP	Bicolor	White	Brown red	Solid flush with defined stripes	Foliaceous	2
Reguard-1	3616 AD	Lérida, SP	Bicolor	Cream	Orange red	Only solid flush	Filiform	2
Reineta Blanca Canadá	308 AD	Zaragoza, SP	Green	Cream	-	-	Foliaceous	3
Reineta Blanca Canadá	3194 AD	Zaragoza, SP	Green	Cream	-	-	Foliaceous	3
Reineta Inesita Asua	2543 AD	Bilbao, SP	Bicolor	White	Red	Only solid flush	Foliaceous	3
Reineta Regil	3466 AD	Vizcaya, SP	Green	Greenish	-	-	Foliaceous	3
Reneta	3408 AD	Mallorca, SP	Bicolor	White	Red	Only solid flush	Filiform	2
Roja Valle Benejama	1038 AD	Valencia, SP	Bicolor	White	Red	Only solid flush	Filiform	2
Roser de la Reula	3552 AD	Lérida, SP	Bicolor	Cream	Red	Solid flush with defined stripes	Filiform	2
Ruixou-1	3614 AD	Lérida, SP	Bicolor	Cream	Orange red	Only solid flush	Filiform	2
San Miguel	2579 AD	La Rioja, SP	Bicolor	Cream	Red	Solid flush with defined stripes	Foliaceous	2
Sandía	3336 AD	Lugo, SP	Bicolor	White	Red	Only stripes	Filiform	3
Sant Joan	3409 AD	Mallorca, SP	Bicolor	Greenish	Orange red	Solid flush with defined stripes	Filiform	2
Santa Margarida	3401 AD	Gerona, SP	Bicolor	Greenish	Red	Only stripes	Foliaceous	3
Signatillis	3403 AD	Gerona, SP	Green	White	-	-	Foliaceous	2
Solafuente	3559 AD	Cantabria, SP	Bicolor	White	Red	Solid flush with defined stripes	Foliaceous	3
Taüll-1	3623 AD	Lérida, SP	Green	Cream	-	-	Filiform	2
Tempera	3334 AD	Lugo, SP	Green	Greenish	-	-	Filiform	2
Terrera	3469 AD	Gerona, SP	Brown	Yellowish	-	-	Foliaceous	3
Toxta	3471 AD	Gerona, SP	Green	White	-	-	Foliaceous	2
Transparente	3377 AD	Navarra, SP	Green	White	-	-	Foliaceous	2
Transparente Blanca	3344 AD	Navarra, SP	Yellow	Greenish	-	-	Foliaceous	2
Urarte	3415 AD	La Rioja, SP	Green	Greenish	-	-	Foliaceous	3
Urtebete	3345 AD	Navarra, SP	Green	Yellowish	-	-	Foliaceous	2
Valsaina	3558 AD	Cantabria, SP	Bicolor	Cream	Red	Solid flush with defined stripes	Filiform	2
Verde Doncella	310 AD	Zaragoza, SP	Bicolor	Cream	Pink red	Only solid flush	Filiform	2
Vinçada Tardia	3621 AD	Lérida, SP	Green	Cream	-	-	Foliaceous	3

SP: Spain

Table 2 Units, minimum, maximum, and mean values for all the traits evaluated for 80 apple accessions

Trait	Units/description	Minimum	Maximum	Maximum/ Minimum value	Mean $\pm$ SE
Full Bloom (FB)	Julian days	81.9	122.5	1.5	102.0 $\pm$ 0.6
Harvest date (HB)	Julian days	175.5	296.2	1.7	247.0 $\pm$ 3.8
Fruit height (FH)	mm	50.2	74.7	1.5	61.6 $\pm$ 0.6
Fruit diameter (FD)	mm	56.3	88.3	1.6	73.0 $\pm$ 0.7
Length of stalk (LS)	mm	6.3	24.0	3.8	12.4 $\pm$ 0.4
Thickness of stalk (TS)	mm	2.3	5.0	2.1	3.4 $\pm$ 0.1
Width of stalk cavity (WS)	mm	23.9	42.0	1.8	32.5 $\pm$ 0.4
Depth of stalk cavity (DS)	mm	8.8	17.7	2.0	12.9 $\pm$ 0.2
Width of eye basin (WE)	mm	20.5	39.5	1.9	29.5 $\pm$ 0.5
Depth of eye basin (DE)	mm	3.9	10.3	2.6	6.9 $\pm$ 0.1
Ratio height / diameter (FH/FD)	Esfericity	0.7	1.1	1.5	0.8 $\pm$ 0.0
Fruit weight (FW)	Grams	77.6	265.8	3.4	161.0 $\pm$ 4.5
Over color (OC)	Percentage	0.0	75.0	-	24.9 $\pm$ 2.9
L* ¹	Lightness	51.5	78.4	1.5	69.1 $\pm$ 0.5
a* ¹	Greenness/redness	-15.2	19.2	-	1.5 $\pm$ 0.5
b* ¹	Blueness/yellowness	228.5	50.5	0.2	40.9 $\pm$ 0.4
C* ¹	Chroma	32.1	66.9	2.1	43.9 $\pm$ 0.5
h* ¹	Lightness' angle	70.1	118.0	1.7	105.7 $\pm$ 1.1
L* ²	Lightness	43.5	79.9	1.8	65.0 $\pm$ 0.9
a* ²	Greenness/redness	-5.8	30.3	-	5.1 $\pm$ 0.9
b* ²	Blueness/yellowness	18.5	56.5	3.0	38.2 $\pm$ 0.8
C* ²	Chroma	28.9	58.4	2.0	41.2 $\pm$ 0.6
h* ²	Lightness' angle	36.9	115.1	3.1	91.7 $\pm$ 2.2
Flesh firmness (FF)	Newtons	47.5	112.4	2.4	72.8 $\pm$ 0.1
Solubles solids content (SSC)	°Brix	10.0	18.1	1.8	13.1 $\pm$ 0.2
Titrateable acidity (TA)	g acid malic L ⁻¹	1.7	17.3	10.3	8.0 $\pm$ 0.4
Ripening index (RI)	SSCT/TA	0.6	8.7	15.0	2.5 $\pm$ 0.2

¹ Color parameters measured on shaded side

² Color parameters measured on blushed side

Table 3 Mean values of agronomic, morphological and quality traits evaluated depending on climate, pomological and genetic traits

Traits	n	FB	HD	FH	FD	LS	TS	WS	DS	WE	DE	FH/FD	FW	FF	SSC	TA	RI
<i>Climate influence</i>																	
Atlantic	12	104.7 a	242.7	60.0 b	73.3 a	11.9	3.5 a	32.3	12.4	30.1	7.1	0.82 b	164.5 b	77.0 a	12.9	9.9 a	1.5 b
Continental	54	102.01 b	246.3	62.9 a	74.1 a	12.6	3.4 ab	32.9	13.1	29.1	6.9	0.85 a	166.2 a	73.1 a	13.1	8.3 b	2.5 a
Mediterranean	14	99.2 c	247.3	58.3 b	69.6 a	12.4	3.3 b	31.4	12.6	29.2	6.9	0.84 ab	133.6 b	68.4 b	13.1	7.8 a	2.8 a
<i>Flesh color</i>																	
Cream	31	104.4 a	260.8 a	62.6 a	73.6 b	11.5 c	3.4 b	32.0 b	12.9 ab	28.8 b	6.7 b	0.85 a	163.6	73.8 b	14.3 a	6.6 b	3.1 a
Greenish	15	102.7 a	229.5 c	60.9 ab	71.2 b	13.3 a	3.4 b	31.7 b	12.3 b	28.3 b	6.8 b	0.86 a	149.9	76.9 a	12.2 b	11.2 a	1.4 b
White	30	98.7 b	239.6 bc	61.4 ab	73.2 b	13.1 ab	3.4 b	33.2 b	13.1 ab	29.8 b	7.2 b	0.84 a	158.3	69.2 b	12.32b	8.4 b	2.4 a
Yellowish	4	105.4 a	250.8 ab	58.9 b	76.1 a	11.8 bc	3.8 a	35.9 a	13.7 a	34.3 a	8.2 a	0.77 b	165.4	79.1 a	13.6 a	10.5 a	1.4 b
<i>Leaf blades stipules</i>																	
Filiform	36	101.3	247.5	60.7 b	69.9 b	13.1 a	3.2 b	30.9 b	12.5 b	27.4 b	6.7	0.87 a	142.5 b	70.9	13.0	6.8 b	3.0 a
Foliaceous	44	102.5	244.7	62.3 a	75.6 a	12.0 b	3.6 a	33.9 a	13.2 a	30.9 a	7.1	0.83 b	175.0 a	74.3	13.1	9.7 a	1.8 b
<i>Genetic traits</i>																	
Diploid	49	101.9	247.0	61.3	71.4 b	12.7 a	3.3 b	31.8 b	12.9	28.6 b	6.9	0.86 a	148.9 b	72.8	12.9	7.7 b	2.7 a
Triploid	21	101.8	242.9	62.5	77.9 a	11.7 b	3.7 a	34.8 a	12.9	31.3 a	7.2	0.80 b	193.2 a	72.5	13.4	10.6 a	1.5 b

n: number of cultivars tested. Means separation within rows by Duncan test ($P \leq 0.05$). In each column. values with the same letter are not significantly different.

FB: full bloom; HD: harvest date; FH: fruit height; FD: fruit diameter; LS: lenght of stalk; TS: thickness of stalk; WS: width of stalk cavity; DS: depth of stalk cavity; WE: width of eye basin; DE: depth of eye basin; FH/FD: sphericity; FW: fruit weight; FF: flesh firmness; SSC: soluble solids content; TA: titratable acidity; RI: ripening index.

Table 4 Mean values of color parameters evaluated depending on fruit type, pattern of over color and hue over color

Traits	n	OC	Shaded side					Blushed side				
			L*	a*	b*	C*	h*	L*	a*	b*	C*	h*
Fruit type												
Bicolor	49	26.8	68.9 b	4.6	40.6 b	43.8 ab	105.1	61.5 c	8.1 a	35.0 b	38.9 b	82.7 b
Green	22	-	69.1 b	1.0	40.9 b	43.3 b	108.4	69.1 b	1.6 b	42.8 a	44.0 a	104.8 a
Yellow	8	-	73.5 a	0.6	42.9 a	45.4 a	107.4	73.9 a	-1.1 b	43.4 a	45.2 a	107.3 a
Pattern of over color												
None over color	31	-	69.7 ab	1.1 ab	41.1 ab	43.4	106.8 ab	69.5 a	1.4 c	42.8 a	44.0 a	105.6 a
Only solid flush	17	28.7 ab	70.4 a	1.2 ab	41.9 a	44.8	106.6 ab	63.8 bc	7.2 ab	37.4 b	41.0 b	84.8 b
Solid flush with defined stripes	16	35.5 a	68.4 b	3.5 a	40.4 abc	43.9	100.7 b	60.0 d	10.8 a	33.8 b	38.1 cd	75.0 c
Only stripes	10	22.7 b	68.0 b	-0.9 b	39.6 bc	42.7	107.7 a	60.9 c	5.8 bc	33.9 b	37.7 d	87.3 b
Flushed and mottled	4	18.0 b	68.5 b	0.8 ab	39.0 c	43.3	111.6 a	65.1 b	2.5 c	37.1 b	40.2 bc	103.2 a
Hue over color												
None	31	-	69.7 ab	1.1 b	41.1 bc	43.4 b	106.8 ab	68.5 a	1.4 b	42.8 a	44.0 a	105.6 a
Orange red	11	13.1 b	70.6 a	-0.5 b	43.4 a	48.2 a	108.8 ab	70.1 a	1.7 b	42.0 a	44.5 a	94.8 b
Pink red	10	11.9 b	71.5 a	4.2 a	42.8 ab	45.9 a	110.8 a	68.6 a	0.2 b	40.1 a	41.1 b	96.6 b
Red	28	33.1 a	68.4 b	1.0 b	39.7 c	42.8 b	103.3 b	59.3 b	10.6 a	33.1 b	37.9 c	73.4 c

n: number of cultivars tested. Means separation within rows by Duncan test ($P \leq 0.05$). In each column, for each trait values with the same letter are not significantly different.

OC: over color; L*: lightness; a*: greenness/redness; b*: blueness/yellowness; C*: chroma; h*: lightness' angle

Table 5 Pearson's correlation between pairs of traits evaluated

Traits	HD	FD	LS	TS	WS	DS	WE	DE	FH/FD	FW	OC	L* ¹	a* ¹	b* ¹	C* ¹	h* ¹	L* ²	a* ²	b* ²	C* ²	h* ²	FF	SSC	TA	RI
Ploidy level	ns	ns	0.39**	-0.10*	0.23**	0.30**	ns	0.25**	0.36**	-0.25**	ns	-0.16*	-0.25**	ns	ns	ns	ns	ns	-0.12*	ns	ns	ns	ns	ns	0.28**
FB	0.35**	ns	ns	ns	-0.28**	ns	ns	ns	ns	ns	ns	-0.18**	ns	ns	ns	-0.24**	-0.16*	0.14*	ns	ns	ns	0.20**	0.31**	ns	ns
HD		-0.11*	-0.21**	ns	-0.19*	0.30**	ns	ns	0.20**	ns	0.26**	ns	0.32**	ns	ns	-0.27**	-0.11*	0.22**	ns	ns	-0.20**	0.32**	0.44**	-0.57**	0.44**
FH		0.53**	ns	0.14**	0.22**	0.47**	ns	0.17*	0.57**	0.69**	-0.18**	ns	ns	ns	ns	0.15**	0.11*	-0.12*	ns	ns	0.17**	-0.11*	ns	ns	0.12*
FD			ns	0.38**	0.84**	0.45**	0.75**	0.45**	-0.37**	0.90**	ns	-0.12*	ns	ns	ns	ns	ns	ns	ns	ns	-0.12*	ns	0.35**	-0.32**	
LS				-0.39**	ns	ns	-0.14*	-0.24**	ns	ns	ns	ns	-0.12*	ns	ns	ns	ns	ns	-0.12*	-0.19**	ns	ns	-0.15**	0.14**	ns
TS					0.41**	0.16*	0.36**	0.19*	-0.21**	0.35**	ns	-0.13*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.12*	-0.12*	
WS						0.41**	0.79**	0.55**	-0.52**	0.82**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	
DS							0.36**	0.50**	ns	0.76**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.65*	
WE								0.64**	-0.57**	0.78**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.40*	-0.35*	
DE									-0.27**	0.66**	ns	0.53*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	
FH/FD										-0.12*	-0.25**	ns	ns	ns	ns	ns	0.15**	ns	0.12*	ns	0.13*	ns	ns	-0.37**	0.45**
FW											ns	-0.16**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.25**	-0.20**
OC												-0.47**	0.20*	-0.52**	-0.51**	-0.58**	-0.71**	0.73**	-0.48**	-0.38**	-0.76**	ns	0.15*	ns	ns
L* ¹													ns	0.47**	0.32**	0.39**	0.62**	-0.24**	0.28**	0.29**	0.28**	-0.11*	-0.12*	-0.15**	ns
a* ¹														-0.21**	-0.21**	-0.32**	ns	0.39**	ns	-0.11*	-0.21**	ns	0.13*	-0.31**	0.18**
b* ¹															0.72**	0.25**	0.41**	-0.35**	0.52**	0.64**	0.25**	ns	0.29**	ns	0.13*
C* ¹																0.34**	0.31**	-0.46**	0.10*	0.58**	0.18**	ns	0.17**	ns	0.11*
h* ¹																	0.34**	-0.58**	-0.15**	ns	0.57**	0.15**	-0.34**	0.16**	-0.12*
L* ²																			-0.67**	0.67**	0.59**	0.81**	-0.18**	ns	ns
a* ²																				-0.30**	-0.38**	-0.78**	ns	ns	-0.24**
b* ²																					0.65**	0.52**	-0.18**	ns	ns
C* ²																						0.39**	-0.14**	0.22**	ns
h* ²																							ns	-0.25**	0.18**
FF																							ns	ns	ns
SSC																								-0.19**	0.25**
TA																									-0.77**

* $P \leq 0.05$, ** $P \leq 0.01$ represent significant values; *ns* not significant

¹ Color parameters measured on shaded side

² Color parameters measured on blushed side

Table 6 Eigenvectors and accumulative variance of the four principal components (PCs) estimated from the 21 variables

Variable	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8
FB	-0.14	0.11	0.49	-0.44	0.46	0.90	-0.45	1.00
HD	-0.29	0.15	1.00	0.17	-0.03	0.14	-0.05	-0.11
FH	0.28	-0.24	0.21	1.00	0.84	0.14	0.01	0.16
FD	0.98	-0.25	0.11	0.05	0.44	-0.07	-0.29	-0.07
LS	-0.24	0.15	-0.66	0.40	0.35	-0.66	0.05	0.66
TS	0.68	-0.24	0.23	-0.41	0.21	1.00	-0.14	-0.17
WS	1.00	-0.20	0.02	-0.03	-0.53	0.00	-0.38	-0.18
DS	0.53	-0.08	0.54	0.61	-1.00	0.42	0.12	-0.29
WE	0.90	-0.07	0.17	-0.36	-0.66	0.59	0.17	-0.01
DE	0.68	-0.14	0.25	0.03	-0.24	-0.02	-0.16	-0.02
FW	0.86	-0.17	0.15	0.30	0.76	-0.08	-0.32	-0.18
OC	0.20	0.81	-0.09	-0.06	-0.20	0.07	-0.60	0.06
L* ¹	-0.45	-0.50	-0.09	0.18	-0.89	-0.21	-0.80	0.32
a* ¹	-0.30	0.37	0.49	0.36	0.11	-0.88	0.03	-0.23
b* ¹	-0.40	-0.59	0.27	-0.27	0.04	0.19	-0.59	-0.38
L* ²	-0.36	-0.83	0.02	0.13	-0.22	-0.15	0.01	0.23
a* ²	0.13	0.82	0.10	0.07	-0.29	-0.25	-0.49	-0.14
b* ²	-0.41	-0.74	0.29	-0.09	-0.02	-0.04	0.04	-0.31
FF	-0.28	0.22	0.73	-0.17	-0.32	0.22	1.00	0.23
SSC	-0.26	0.03	0.64	-0.58	0.69	0.33	-0.24	-0.80
TA	0.56	-0.18	-0.61	-0.46	0.18	0.25	0.56	-0.25
Accumulated variance %	24	44	58	65	72	77	81	85

¹ Color parameters measured on shaded side

² Color parameters measured on blushed side

Fig. 1 Classification of the 80 apple accessions in six clusters by the four main origins of variability corresponding to the fourth principal components of a PCA with 21 variables.

Fig. 2 Two accessions (A: Astrakan Roja; B: Landetxo) with identical SSR profile (Urrestarazu et al. 2012) and different fruit phenotypic characteristics (pattern over color and ribbing).

ELECTRONIC SUPPLEMENTARY MATERIAL

Phenotypic diversity of Spanish apple (*Malus x domestica* Borkh) accessions grown at the vulnerable climatic conditions of the Ebro Valley, Spain

Scientia Horticulturae

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Online Resource 1 The average annual temperature daily temperature (Tm), the average annual maximum daily temperature (Tmax), and the average annual minimum daily temperature (Tmin) values for 2003-2013 period.

Year	Tm	Tmax	Tmin
2003	15.3	22.0	9.0
2004	14.4	21.2	8.4
2005	14.1	21.1	7.7
2006	15.1	21.8	8.8
2007	14.1	21.2	7.6
2008	14.2	21.0	8.0
2009	15.0	22.3	8.5
2010	14.1	20.9	8.0
2011	15.4	22.6	8.9
2012	15.1	22.3	8.4
2013	14.3	20.9	8.2

Online Resource 2 Mean values of full bloom (FB) and harvest date (HD) for each accession and year of study.

Accessions	Accession number	Full bloom (Julian days)							Harvest date (Julian days)										
		2003	2008	2009	2010	2011	2012	2013	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Acipres	3339 AD	108	105	104		100	107	109	280	282		283	288	288				282	288
Almenar-2	3555 AD		98	98	112	100	103	112						281			264	250	248
Ascara 1	3423 AD	105	106	98		99	103	114	247	225		233	250	232				236	235
Ascara 2	3424 AD	104	106	103		99	103	114	241	233		234	246	232				236	235
Astrakán roja	3378 AD	126	92	83		91	94	100	182	189		195	179	172	174			180	186
Audiena de Oroz	3375 AD	100	98	96		96	98	106	100					98	96		96	98	106
Bellaguardia Lardero	3547 AD		91	93	105	94	103	103						91	93	105	94	103	103
Boluaga	3340 AD	114	113	108		100	117	115	114					113	108		100	117	115
Bossost-1	3626 AD		93	96	113	97	106	107						93	96	113	97	106	107
Bossost-4	3629 AD		97	98	111	103	100	110						97	98	111	103	100	110
Bossost-5	3630 AD		98		111	96	100	106						98		111	96	100	106
Bost Kantoia	3341 AD	102	101	96		95	103	107	102					101	96		95	103	107
Cabdellá-2	3613 AD		106	98	116	95	111	112						106	98	116	95	111	112
Calvilla de San Salvador	3342 AD	101	92	90		95	100	104	101					92	90		95	100	104
Camosa	3620 AD		116	106	122	104	111	114						116	106	122	104	111	114
Camuesa del Llobregat	1342 AD	102	97	93	110	95	101	106	102					97	93	110	95	101	106
Camuesa fina d'Aragón	3372 AD	109	101	98		98	97	108	109					101	98		98	97	108
Cella	2512 AD		127	119	121	116	117	135						127	119	121	116	117	
Ciri Blanc	3402 AD	110	103	107		100	111	114	135	110					103	107		100	111
Cirio	3615 AD		106	98	110	99	103	108						106	98	110	99	103	108
Cul de Cirio	3551 AD		105	103	115	99	111	114						105	103	115	99	111	114
De Agosto	3619 AD			103	120	102	117	114							103	120	102	117	114
De pera	3416 AD	111	108	98		98	107	113	111					108	98		98	107	113
Del ciri	3413 AD	107	101	72		97	100	109	107					101	72		97	100	109
Esperiega	3420 AD	112	115	110		102	109	114	112					115	110		102	109	114
Eugenia	3468 AD	112	113	103		105	116	123	112					113	103		105	116	123
Guillemes	3411 AD	101	96	90		96	100	105	101					96	90		96	100	105
Helada	3368 AD	101	93	96		96	100	105	101					93	96		96	100	105
Hierro	3374 AD	102	98	96		96	104	107	102					98	96		96	104	107
Irgo-2	3622 AD		93	90	105	94	99	106						93	90	105	94	99	106
Landetxo	3343 AD	99	92	83		92	98	98	99					92	83		92	98	98
Les-1	3624 AD		98	98	105	96	103	107						98	98	105	96	103	107
Les-2	3625 AD		99	98	110	96	103	106						99	98	110	96	103	106
Mañaga	3554 AD		95	97	105	95	100	108						95	97	105	95	100	108
Mañaga	469 AD	100	95	90	100	96	101	108	100					95	90	100	96	101	108
Marinera	3412 AD	81	74	82	85	83	84	84	81					74	82	85	83	84	84
Marquinez	3419 AD	100	93	93		93	100	109	100					93	93		93	100	109
Montcada-1	3631 AD		106	98	112	98	103	112						106	98	112	98	103	112
Morro de Liebre	3256 AD	98	91	86	109	92	101	97	98					91	86	109	92	101	97

Online Resource 2 Continued

Accessions	Accession number	Full bloom (Julian days)							Harvest date (Julian days)										
		2003	2008	2009	2010	2011	2012	2013	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Navalmoral de Bejar-1	3548 AD		98	98	115	97	103	112						98	98	115	97	103	112
Nesple	3410 AD	102	98	96		95	103	106	102					98	96		95	103	106
Normanda	3252 AD		92	96	105	95	101	107						92	96	105	95	101	107
Ortell	413 AD	92	91	93	96	96	101	103	92					91	93	96	96	101	103
Ortell	3546 AD		98	96	105	94	100	102						98	96	105	94	100	102
Pera 2	3417 AD	110	111	98		98	107	113	110					111	98		98	107	113
Pera de Sangüesa	3379 AD	110	106	96		99	103	109	110					106	96		99	103	109
Pero pardo	3369 AD	106	106	98		98	103	108	106					106	98		98	103	108
Peromingán	1158 AD	105	111	96	111	98	104	110	105					111	96	111	98	104	110
Peruco de Caparroso	3373 AD	87	91	86		91	107	100	87					91	86		91	107	100
Poma de San Juan	3556 AD		91	86	102	94	95	102						91	86	102	94	95	102
Prau Riu 3	3491 AD		106	98	116	99	101	107						106	98	116	99	101	107
Prau Riu 4	3492 AD		106	93	105	95	100	106						106	93	105	95	100	106
Prau Riu 5	3493 AD		113	103	116	103	111	115						113	103	116	103	111	115
Rebellón	3370 AD	108	113	108		97	107	108	108					113	108		97	107	108
Reguard-1	3616 AD		96	98	105	94	100	106						96	98	105	94	100	106
Reineta Blanca Canadá	308 AD	105	100	97	105	96	101	108	105					100	97	105	96	101	108
Reineta Blanca Canadá	3194 AD	105	102	97	112	97	103	109	105					102	97	112	97	103	109
Reineta Inesita Asua	2543 AD		100	86	105	94	98	103						100	86	105	94	98	103
Reineta Regil	3466 AD	112	115	114		103	109	116	112					115	114		103	109	116
Reneta	3408 AD	100	98	90		96	100	106	100					98	90		96	100	106
Roja Valle Benejama	1038 AD	102	95	93	105	96	101	109	102					95	93	105	96	101	109
Roser de la Reula	3552 AD		93	90	110	94	100	103						93	90	110	94	100	103
Ruixou-1	3614 AD		92	86	110	95	98	107						92	86	110	95	98	107
San Miguel	2579 AD	110	116	110	118	104	107	124	110					116	110	118	104	107	124
Sandía	3336 AD	109	108	107		98	111	114	109					108	107		98	111	114
Sant Joan	3409 AD	98	96	86		91	100	103	98					96	86		91	100	103
Santa Margarida	3401 AD	102	98	90		95	101	105	102					98	90		95	101	105
Signatillis	3403 AD	106	99	98		96	96	108	106					99	98		96	96	108
Solafuente	3559 AD		93	93	105	94	100	106						93	93	105	94	100	106
Taüll-1	3623 AD		106	98	110	97	105	114						106	98	110	97	105	114
Tempera	3334 AD	110	104	103		99	107	110	110					104	103		99	107	110
Terrera	3469 AD	99	93	93		93	96	100	99					93	93		93	96	100
Toxta	3471 AD	104	113	106		96	103	112	104					113	106		96	103	112
Transparente	3377 AD	98	92	83		91	93	100	98					92	83		91	93	100
Transparente Blanca	3344 AD	100	93	86		95	98	103	100					93	86		95	98	103
Urarte	3415 AD	100	93	90		93	103	104	100					93	90		93	103	104
Urtebete	3345 AD	113	113	110		100	109	114	113					113	110		100	109	114
Valsaina	3558 AD		97	96	110	97	111	106						97	96	110	97	111	106
Verde Doncella	310 AD	100	98	90	110	95	101	106	100					98	90	110	95	101	106
Vinçada Tardia	3621 AD		96	98	105	96	103	108						96	98	105	96	103	108

From 2004 to 2007, no data for FB are available.

Online Resource 3 Apple accessions maintained at the Experimental Station of Aula Dei (CSIC-Zaragoza, Spain). Collection information includes name and bank number and mean values of each trait evaluated in this study.

Accessions	Accession number	FB		HD		FH		FD		FH/FD		FW		LS		TS		WS		DS	
Acipres	3339 AD	105.50	± 1.23	284.43	± 1.22	65.56	± 1.64	74.50	± 1.51	0.88	± 0.01	176.26	± 16.29	14.80	± 1.75	2.68	± 0.24	32.37	± 2.12	13.46	± 1.38
Almenar-2	3555 AD	103.83	± 2.47	260.75	± 6.69	63.95	± 4.06	74.35	± 3.76	0.87	± 0.03	211.06	± 38.98	10.46	± 2.07	4.03	± 0.43	30.44	± 1.95	14.28	± 1.87
Ascara 1	3423 AD	104.17	± 2.17	236.86	± 3.08	55.75	± 1.44	75.14	± 1.66	0.74	± 0.01	182.47	± 19.73	13.89	± 1.64	3.45	± 0.30	33.08	± 2.37	11.57	± 1.17
Ascara 2	3424 AD	104.83	± 1.89	236.71	± 1.77	52.08	± 1.49	67.26	± 1.90	0.78	± 0.02	129.56	± 15.53	14.79	± 2.09	3.01	± 0.33	33.30	± 2.65	12.26	± 0.98
Astrakán roja	3378 AD	97.67	± 5.61	182.13	± 2.55	57.82	± 1.82	72.43	± 2.04	0.80	± 0.02	145.40	± 16.98	14.82	± 2.09	3.09	± 0.23	34.20	± 2.71	10.31	± 1.41
Audiena de Oroz	3375 AD	99.00	± 1.41	270.67	± 2.92	69.48	± 3.24	88.03	± 3.37	0.79	± 0.02	265.77	± 38.43	10.94	± 1.59	4.84	± 0.59	39.67	± 1.67	15.41	± 2.62
Bellaguardia Lardero	3547 AD	98.17	± 2.31	178.33	± 1.79	58.32	± 4.29	70.08	± 3.45	0.83	± 0.03	145.19	± 28.23	13.47	± 2.33	3.51	± 0.40	31.75	± 2.54	9.11	± 1.19
Boluaga	3340 AD	111.17	± 2.35	253.50	± 3.11	59.38	± 2.15	79.87	± 3.25	0.75	± 0.02	166.65	± 28.59	7.04	± 0.95	5.03	± 0.65	36.70	± 1.96	11.74	± 1.33
Bossost-1	3626 AD	102.00	± 2.93	208.29	± 4.44	67.19	± 3.25	80.38	± 2.90	0.84	± 0.03	219.67	± 28.39	11.85	± 1.88	3.59	± 0.32	33.49	± 1.59	11.46	± 1.58
Bossost-4	3629 AD	103.17	± 2.27	291.33	± 4.80	53.85	± 2.18	70.44	± 2.19	0.77	± 0.02	141.80	± 11.75	6.82	± 0.98	3.75	± 0.32	31.78	± 1.41	12.57	± 1.40
Bossost-5	3630 AD	102.20	± 2.50	212.33	± 4.99	61.48	± 2.07	82.75	± 2.25	0.75	± 0.02	232.93	± 35.24	9.52	± 2.76	4.25	± 0.46	38.66	± 2.69	12.51	± 1.82
Bost Kantoia	3341 AD	100.67	± 1.69	272.43	± 1.09	61.09	± 2.17	76.82	± 2.43	0.80	± 0.02	182.00	± 30.79	8.73	± 1.41	3.44	± 0.30	34.28	± 2.90	15.31	± 1.39
Cabdellá-2	3613 AD	106.33	± 3.12	226.14	± 4.29	61.23	± 2.91	77.99	± 3.23	0.78	± 0.02	205.27	± 34.68	12.38	± 1.72	3.23	± 0.21	33.56	± 2.12	13.86	± 1.38
Calvilla de San Salvador	3342 AD	97.00	± 2.07	206.29	± 3.65	67.45	± 2.69	75.86	± 2.22	0.89	± 0.02	176.59	± 23.68	16.49	± 2.00	3.34	± 0.24	36.17	± 2.11	15.37	± 1.39
Camosa	3620 AD	112.17	± 2.50	245.00	± 4.57	64.19	± 4.24	68.00	± 4.05	0.94	± 0.03	121.11	± 21.42	20.86	± 2.37	2.87	± 0.32	27.06	± 1.94	12.01	± 2.21
Camuesa del Llobregat	1342 AD	100.57	± 2.14	272.40	± 3.80	53.75	± 1.75	66.92	± 2.14	0.81	± 0.02	119.91	± 13.44	18.27	± 2.38	2.80	± 0.31	30.65	± 1.88	11.90	± 1.66
Camuesa fina d'Aragón	3372 AD	101.83	± 2.01	224.29	± 3.04	72.35	± 2.83	84.48	± 2.79	0.85	± 0.03	215.49	± 26.13	10.31	± 1.08	4.53	± 0.78	41.95	± 4.36	15.97	± 1.90
Cella	2512 AD	122.50	± 2.73	269.29	± 1.82	55.90	± 2.06	67.44	± 2.59	0.83	± 0.02	110.80	± 12.64	11.51	± 1.16	3.47	± 0.30	31.37	± 3.67	12.08	± 1.51
Ciri Blanc	3402 AD	107.50	± 1.97	279.17	± 3.93	70.25	± 2.99	64.29	± 2.89	1.09	± 0.03	103.72	± 19.37	11.13	± 1.49	3.10	± 0.46	29.28	± 1.40	14.53	± 2.46
Cirio	3615 AD	104.00	± 1.83	262.00	± 1.45	61.61	± 5.42	77.08	± 4.79	0.79	± 0.03	175.02	± 38.01	10.38	± 2.18	3.01	± 0.41	32.39	± 1.74	11.10	± 1.72
Cul de Cirio	3551 AD	107.83	± 2.43	271.50	± 6.61	74.72	± 2.91	67.48	± 1.73	1.10	± 0.03	162.73	± 18.50	10.42	± 1.22	3.36	± 0.27	24.71	± 1.84	12.92	± 1.45
De Agosto	3619 AD	111.20	± 3.32	242.33	± 0.55	62.26	± 3.37	67.44	± 3.13	0.91	± 0.02	135.69	± 21.79	23.93	± 1.77	3.02	± 0.18	26.16	± 1.92	9.95	± 1.28
De pera	3416 AD	105.83	± 2.42	272.43	± 2.02	65.61	± 2.51	73.52	± 2.43	0.90	± 0.02	148.64	± 19.08	11.41	± 1.53	3.75	± 0.28	33.71	± 3.25	15.20	± 2.02
Del ciri	3413 AD	97.67	± 5.02	280.00	± 2.71	71.82	± 2.21	65.62	± 2.38	1.10	± 0.03	137.37	± 19.93	14.36	± 2.01	3.11	± 0.39	25.15	± 1.92	11.65	± 1.88
Esperiega	3420 AD	110.33	± 1.76	278.17	± 2.13	60.22	± 3.52	73.04	± 2.17	0.83	± 0.06	153.86	± 18.86	8.55	± 1.00	3.22	± 0.38	34.12	± 1.60	11.86	± 1.43
Eugenia	3468 AD	112.00	± 2.75	266.80	± 3.47	50.68	± 2.00	59.87	± 2.27	0.85	± 0.01	78.78	± 9.03	15.57	± 1.39	2.73	± 0.22	25.36	± 1.21	11.10	± 1.14
Guillemes	3411 AD	98.00	± 1.95	274.86	± 1.90	59.60	± 1.85	69.86	± 1.82	0.85	± 0.02	127.28	± 8.95	8.75	± 1.06	3.45	± 0.28	30.57	± 1.90	15.39	± 2.50
Helada	3368 AD	98.50	± 1.63	281.86	± 2.76	60.16	± 2.03	72.27	± 2.73	0.83	± 0.02	129.05	± 19.32	10.78	± 1.27	2.95	± 0.22	31.58	± 2.69	14.91	± 1.45
Hierro	3374 AD	100.50	± 1.71	287.86	± 1.90	58.44	± 1.69	73.82	± 1.66	0.79	± 0.02	165.30	± 17.30	11.55	± 1.07	3.24	± 0.34	33.32	± 2.07	14.03	± 1.64
Irgo-2	3622 AD	97.83	± 2.49	291.50	± 3.59	65.97	± 2.35	69.85	± 1.55	0.94	± 0.03	157.34	± 12.58	9.68	± 1.20	3.25	± 0.27	27.24	± 2.02	11.63	± 1.77
Landetxo	3343 AD	93.67	± 2.29	202.00	± 13.37	56.71	± 1.96	72.90	± 2.20	0.78	± 0.01	143.08	± 15.93	16.50	± 1.59	3.07	± 0.20	31.46	± 1.43	10.62	± 0.91
Les-1	3624 AD	101.17	± 1.67	199.75	± 1.77	59.96	± 2.27	74.34	± 1.98	0.81	± 0.02	153.26	± 17.14	7.49	± 1.54	4.49	± 0.33	34.81	± 2.78	11.62	± 1.10
Les-2	3625 AD	102.00	± 2.00	215.80	± 5.57	57.67	± 1.78	78.50	± 2.38	0.73	± 0.02	183.82	± 18.64	10.05	± 1.21	4.15	± 0.35	33.84	± 1.77	13.13	± 1.60
Mañaga	3554 AD	100.00	± 2.04	280.50	± 2.85	69.52	± 3.23	68.32	± 2.25	1.02	± 0.04	145.70	± 20.51	10.27	± 1.21	3.15	± 0.20	28.24	± 1.61	10.63	± 2.02
Mañaga	469 AD	98.57	± 1.99	286.43	± 1.95	65.85	± 2.10	63.77	± 2.30	1.03	± 0.03	108.48	± 14.27	11.03	± 1.51	2.81	± 0.24	28.44	± 0.86	12.20	± 1.46
Marinera	3412 AD	81.86	± 1.31	175.50	± 2.18	56.88	± 1.51	69.46	± 1.49	0.82	± 0.02	123.49	± 8.46	11.02	± 1.48	2.99	± 0.19	31.96	± 1.84	10.98	± 1.43
Marquinez	3419 AD	98.00	± 2.40	203.86	± 2.22	63.72	± 2.98	80.18	± 2.77	0.79	± 0.03	198.06	± 34.05	11.84	± 1.49	3.33	± 0.24	37.24	± 3.68	14.54	± 2.27
Montcada-1	3631 AD	104.83	± 2.38	251.50	± 6.07	66.83	± 2.44	66.27	± 2.88	1.03	± 0.05	132.10	± 12.82	9.00	± 1.38	3.11	± 0.32	28.59	± 2.49	11.92	± 1.34
Morro de Liebre	3256 AD	96.29	± 2.65	255.50	± 6.27	70.08	± 1.92	76.91	± 1.55	0.91	± 0.02	200.81	± 15.96	16.74	± 1.25	3.41	± 0.21	36.40	± 3.16	15.75	± 1.88
Navalmoral de Bejar-1	3548 AD	103.83	± 2.94	223.50	± 11.01	65.61	± 3.41	77.05	± 3.85	0.85	± 0.02	161.60	± 31.51	10.23	± 1.42	3.39	± 0.25	35.42	± 4.18	14.10	± 2.07
Nesple	3410 AD	100.00	± 1.63	267.50	± 14.56	62.57	± 2.23	76.55	± 1.95	0.82	± 0.02	199.44	± 19.35	12.37	± 1.01	2.73	± 0.21	34.75	± 2.13	15.31	± 1.75
Normanda	3252 AD	99.33	± 2.24	284.50	± 4.54	64.86	± 2.22	74.80	± 2.23	0.87	± 0.03	184.08	± 25.38	10.97	± 1.36	3.29	± 0.33	33.75	± 2.39	14.72	± 1.49
Ortell	413 AD	99.17	± 1.51	296.20	± 3.10	62.97	± 2.89	68.30	± 1.72	0.93	± 0.04	146.29	± 11.02	15.38	± 1.61	2.49	± 0.25	31.85	± 2.18	14.52	± 0.99

Online Resource 3 Continued

Accessions	Accession number	FB		HD		FH		FD		FH/FD		FW		LS		TS		WS		DS	
Ortell	3546 AD	96.00	± 1.60	267.17	± 2.17	62.34	± 1.89	66.16	± 1.95	0.94	± 0.02	109.26	± 11.77	13.73	± 1.35	2.52	± 0.17	32.18	± 1.96	16.02	± 1.43
Pera 2	3417 AD	106.17	± 2.49	270.80	± 3.52	65.92	± 2.31	73.32	± 2.23	0.90	± 0.02	141.61	± 18.66	11.67	± 1.43	3.89	± 0.33	31.98	± 2.17	15.24	± 1.89
Pera de Sangüesa	3379 AD	103.83	± 2.09	275.00	± 2.10	69.54	± 2.11	79.27	± 2.10	0.88	± 0.02	205.15	± 26.31	11.25	± 1.58	3.35	± 0.26	31.88	± 2.51	12.69	± 1.44
Pero pardo	3369 AD	103.17	± 1.62	280.14	± 1.67	68.38	± 1.75	78.07	± 1.92	0.88	± 0.02	197.53	± 22.65	12.41	± 1.88	3.13	± 0.34	33.49	± 2.00	13.51	± 1.87
Peromingán	1158 AD	105.00	± 2.17	239.00	± 5.34	69.34	± 2.19	67.04	± 2.14	1.03	± 0.03	130.14	± 15.79	14.02	± 1.42	3.18	± 0.23	27.28	± 1.86	11.36	± 0.81
Peruco de Caparroso	3373 AD	93.67	± 3.08	193.71	± 2.44	74.44	± 2.67	74.63	± 3.02	1.00	± 0.04	181.57	± 22.97	16.17	± 1.79	3.34	± 0.19	35.24	± 1.87	14.24	± 1.49
Poma de San Juan	3556 AD	95.00	± 2.35	183.80	± 1.75	53.04	± 2.83	56.25	± 2.87	0.95	± 0.03	77.56	± 11.36	15.45	± 1.98	2.34	± 0.25	23.88	± 1.94	8.98	± 2.43
Prau Riu 3	3491 AD	104.50	± 2.52	268.40	± 8.27	59.79	± 2.21	68.13	± 2.38	0.88	± 0.02	150.48	± 17.98	14.66	± 1.68	3.39	± 0.32	31.78	± 3.13	13.48	± 1.53
Prau Riu 4	3492 AD	100.83	± 2.17	234.43	± 6.16	59.61	± 1.95	81.03	± 2.08	0.74	± 0.02	204.75	± 17.35	14.98	± 3.63	3.89	± 0.34	33.54	± 2.81	9.02	± 0.72
Prau Riu 5	3493 AD	110.17	± 2.18	226.00	± 3.27	57.85	± 2.18	71.24	± 2.44	0.81	± 0.01	149.19	± 17.85	11.38	± 1.18	3.23	± 0.25	28.59	± 1.75	11.55	± 1.30
Rebellón	3370 AD	106.83	± 1.98	250.75	± 4.95	62.59	± 1.96	74.39	± 2.09	0.84	± 0.01	194.87	± 15.79	20.95	± 1.89	2.82	± 0.22	31.62	± 2.34	13.09	± 1.58
Reguard-1	3616 AD	99.83	± 1.82	265.00	± 5.42	65.34	± 3.20	68.13	± 3.45	0.94	± 0.03	137.19	± 12.33	10.69	± 1.52	3.37	± 0.26	27.13	± 2.14	12.33	± 1.48
Reineta Blanca Canadá	308 AD	101.71	± 1.57	267.43	± 4.69	60.67	± 2.28	79.91	± 2.71	0.77	± 0.02	195.67	± 29.85	9.06	± 1.02	3.77	± 0.33	37.80	± 3.40	13.47	± 1.46
Reineta Blanca Canadá	3194 AD	103.57	± 1.99	264.83	± 4.70	61.69	± 1.96	81.20	± 2.02	0.76	± 0.02	213.11	± 19.54	10.41	± 1.33	3.69	± 0.29	35.17	± 2.24	14.53	± 1.20
Reineta Inesita Asua	2543 AD	97.67	± 2.59	226.25	± 8.00	63.69	± 2.61	78.47	± 2.78	0.80	± 0.02	190.45	± 26.37	12.22	± 2.25	3.08	± 0.30	33.54	± 2.60	12.05	± 1.91
Reineta Regil	3466 AD	111.50	± 1.82	275.00	± 1.75	53.70	± 2.43	65.96	± 2.76	0.81	± 0.02	115.58	± 19.51	6.31	± 1.17	4.26	± 0.38	28.00	± 1.82	10.50	± 1.91
Reneta	3408 AD	98.33	± 1.98	284.43	± 1.22	50.20	± 1.84	66.53	± 2.35	0.75	± 0.01	90.36	± 7.35	9.91	± 1.59	3.84	± 0.43	32.32	± 2.08	11.22	± 1.19
Roja Valle Benejama	1038 AD	100.14	± 2.04	279.50	± 1.23	50.63	± 1.68	66.62	± 2.14	0.76	± 0.02	91.14	± 10.80	10.17	± 1.23	3.46	± 0.35	30.93	± 1.67	12.38	± 2.09
Roser de la Reula	3552 AD	98.33	± 2.80	244.14	± 6.49	52.36	± 1.79	64.89	± 2.01	0.80	± 0.01	147.22	± 31.63	16.30	± 1.92	2.80	± 0.24	26.63	± 1.72	12.10	± 1.78
Ruixou-1	3614 AD	98.00	± 3.42	275.20	± 2.01	61.89	± 2.56	66.16	± 1.99	0.94	± 0.03	150.93	± 8.74	12.15	± 1.54	3.32	± 0.29	27.46	± 1.22	11.13	± 1.45
San Miguel	2579 AD	112.71	± 2.45	260.86	± 11.85	63.09	± 2.16	79.72	± 2.82	0.79	± 0.02	206.80	± 43.39	9.66	± 1.04	3.73	± 0.32	35.36	± 2.04	12.66	± 1.80
Sandía	3336 AD	107.83	± 2.04	213.33	± 1.38	63.94	± 2.29	73.25	± 2.24	0.87	± 0.01	180.53	± 25.10	14.57	± 1.18	3.40	± 0.27	33.80	± 2.70	15.03	± 2.14
Sant Joan	3409 AD	95.67	± 2.34	196.25	± 4.60	57.14	± 2.70	70.20	± 3.67	0.82	± 0.02	125.93	± 23.82	12.15	± 1.60	3.78	± 0.27	31.12	± 2.84	11.60	± 2.02
Santa Margarida	3401 AD	98.50	± 2.03	190.50	± 1.88	57.66	± 1.35	67.94	± 1.74	0.85	± 0.02	125.85	± 9.75	15.21	± 1.66	3.40	± 0.21	31.99	± 2.21	11.88	± 1.72
Signatillis	3403 AD	100.50	± 1.96	208.29	± 2.17	62.71	± 3.54	82.17	± 2.07	0.76	± 0.03	200.81	± 26.88	12.97	± 1.71	3.44	± 0.31	39.48	± 3.16	13.60	± 1.54
Solafuente	3559 AD	98.50	± 2.26	251.33	± 5.22	65.69	± 2.35	80.04	± 1.61	0.82	± 0.02	236.02	± 17.87	12.20	± 1.28	4.16	± 0.42	37.17	± 2.01	14.55	± 1.70
Taüll-1	3623 AD	105.00	± 2.49	295.75	± 4.53	62.01	± 3.81	72.25	± 4.57	0.87	± 0.02	149.94	± 29.70	12.53	± 1.15	3.08	± 0.21	33.30	± 3.90	15.12	± 2.72
Tempera	3334 AD	105.50	± 1.63	227.14	± 1.72	51.04	± 1.88	66.48	± 1.91	0.77	± 0.02	114.12	± 12.92	15.06	± 1.66	2.49	± 0.20	29.96	± 2.52	12.74	± 2.31
Terrera	3469 AD	95.67	± 1.20	251.50	± 5.09	63.79	± 2.48	79.19	± 2.46	0.80	± 0.02	206.42	± 31.55	13.03	± 1.72	3.95	± 0.34	36.42	± 2.67	13.13	± 1.68
Toxta	3471 AD	105.67	± 2.36	263.43	± 2.35	51.48	± 1.81	69.58	± 1.81	0.74	± 0.01	118.48	± 15.11	9.53	± 1.06	3.50	± 0.23	30.51	± 1.55	12.82	± 1.73
Transparente	3377 AD	92.83	± 2.25	200.67	± 3.93	55.93	± 2.29	73.18	± 2.37	0.76	± 0.02	135.27	± 18.60	11.15	± 1.29	3.63	± 0.29	33.45	± 2.00	12.18	± 1.54
Transparente Blanca	3344 AD	95.83	± 2.25	180.25	± 2.92	58.92	± 2.43	73.30	± 2.52	0.80	± 0.02	135.51	± 17.61	17.57	± 1.98	3.96	± 0.53	35.78	± 3.82	12.16	± 1.65
Urarte	3415 AD	97.17	± 2.22	195.67	± 2.15	68.66	± 3.11	88.28	± 3.67	0.78	± 0.02	261.08	± 38.07	14.78	± 2.45	4.27	± 0.42	39.30	± 3.82	12.76	± 2.73
Urtebete	3345 AD	109.83	± 1.95	262.29	± 2.46	61.66	± 2.37	78.80	± 3.09	0.77	± 0.02	171.69	± 30.08	12.39	± 1.10	3.58	± 0.37	37.89	± 1.61	17.67	± 1.43
Valsaina	3558 AD	102.83	± 2.62	224.14	± 2.96	56.87	± 2.43	71.29	± 2.26	0.80	± 0.02	151.51	± 17.47	10.27	± 1.85	3.39	± 0.22	32.03	± 2.24	11.87	± 2.86
Verde Doncella	310 AD	100.00	± 2.35	265.43	± 12.29	59.96	± 2.64	74.10	± 3.21	0.81	± 0.02	165.42	± 30.41	8.69	± 0.73	3.48	± 0.26	35.18	± 4.47	14.99	± 2.01
Vinçada Tardia	3621 AD	101.00	± 1.90	241.67	± 1.84	62.41	± 3.34	82.46	± 3.34	0.75	± 0.02	218.19	± 42.88	7.46	± 1.16	4.51	± 0.26	39.11	± 2.77	13.55	± 1.13

FB: full bloom; HD: harvest date; FH: fruit height; FD: fruit diameter; LS: lenght of stalk; TS: thickness of stalk; WS: width of stalk cavity; DS: depth of stalk cavity; FH/FD: sphericity; FW: fruit weight.

Online Resource 4 Apple accessions maintained at the Experimental Station of Aula Dei (CSIC-Zaragoza, Spain). Collection information includes name and bank number and mean values of each trait evaluated in this study.

Accessions	Accession number	WE		DE		L (shaded)		a (shaded)		b (shaded)		C (shaded)		h (shaded)		L (blushed)		a (blushed)		b (blushed)	
Acipres	3339 AD	30.44	± 1.53	6.02	± 0.52	66.03	± 1.50	1.48	± 3.16	40.99	± 2.03	43.24	± 1.86	97.12	± 5.97	50.35	± 2.29	21.71	± 4.10	24.92	± 2.69
Almenar-2	3555 AD	29.34	± 2.39	7.59	± 1.72	70.61	± 1.56	3.29	± 2.97	47.05	± 3.10	69.06	± 16.67	86.15	± 17.31	67.63	± 2.82	2.67	± 4.85	44.13	± 4.66
Ascara 1	3423 AD	32.49	± 1.65	6.70	± 0.86	69.10	± 1.38	3.75	± 4.86	35.04	± 0.97	39.07	± 1.33	100.71	± 9.37	59.19	± 3.70	14.07	± 7.78	28.21	± 2.68
Ascara 2	3424 AD	31.51	± 1.66	7.54	± 0.79	68.80	± 1.57	2.39	± 3.36	38.15	± 1.70	40.36	± 1.73	102.20	± 6.12	55.71	± 3.46	12.32	± 5.73	29.16	± 3.19
Astrakán roja	3378 AD	31.67	± 1.65	5.45	± 0.64	69.29	± 1.46	0.78	± 4.22	38.45	± 1.73	41.79	± 1.70	110.47	± 3.22	60.88	± 4.40	5.58	± 5.54	32.21	± 1.82
Audiena de Oroz	3375 AD	39.50	± 2.63	8.75	± 1.43	70.56	± 0.66	5.83	± 0.43	34.77	± 1.27	37.69	± 1.06	114.76	± 0.88	70.81	± 0.79	4.79	± 1.83	34.69	± 0.96
Bellaguardia Lardero	3547 AD	26.91	± 1.26	4.73	± 0.85	77.96	± 1.77	3.22	± 2.24	41.33	± 1.64	43.76	± 1.92	108.51	± 1.47	79.41	± 1.24	-4.03	± 2.58	42.42	± 1.60
Boluaga	3340 AD	36.11	± 3.41	8.84	± 1.07	65.26	± 1.22	-2.99	± 5.11	39.80	± 1.17	42.96	± 0.96	109.78	± 3.94	54.35	± 2.78	7.97	± 4.92	29.15	± 3.45
Bossost-1	3626 AD	28.62	± 1.96	8.37	± 1.95	68.16	± 1.15	2.33	± 4.76	41.46	± 1.20	45.14	± 1.13	112.44	± 2.18	64.66	± 1.98	7.25	± 3.61	36.62	± 1.99
Bossost-4	3629 AD	32.71	± 1.39	8.78	± 1.01	70.35	± 0.96	8.03	± 0.65	45.11	± 1.13	48.73	± 0.97	111.90	± 1.03	67.96	± 2.51	2.25	± 3.74	41.11	± 3.52
Bossost-5	3630 AD	33.77	± 2.39	7.08	± 1.28	68.24	± 1.31	4.65	± 3.03	45.14	± 2.34	47.91	± 2.09	108.82	± 2.87	62.53	± 2.29	3.09	± 1.94	38.25	± 3.69
Bost Kantoia	3341 AD	34.22	± 2.33	9.22	± 1.00	73.40	± 0.84	-0.40	± 4.18	44.80	± 1.02	46.77	± 0.82	106.14	± 3.07	73.39	± 0.94	0.88	± 3.47	44.74	± 0.96
Cabdellá-2	3613 AD	37.28	± 2.40	8.37	± 1.27	66.01	± 2.09	2.62	± 5.84	37.57	± 2.68	40.91	± 3.09	93.54	± 9.64	47.08	± 2.58	28.83	± 4.31	20.07	± 2.30
Calvilla de San Salvador	3342 AD	33.15	± 2.41	9.56	± 1.14	65.40	± 0.68	-15.19	± 5.51	39.26	± 0.65	44.22	± 0.67	118.01	± 0.56	62.99	± 2.62	-5.79	± 6.53	36.01	± 2.77
Camosa	3620 AD	24.04	± 1.64	5.86	± 1.14	71.68	± 2.05	1.53	± 3.29	43.64	± 2.88	45.71	± 2.95	105.58	± 3.22	69.91	± 3.41	4.53	± 3.41	40.63	± 3.65
Camuesa del Llobregat	1342 AD	27.12	± 2.11	6.25	± 0.80	74.06	± 1.56	-0.83	± 2.48	42.59	± 1.01	43.86	± 0.70	102.07	± 3.38	72.41	± 1.93	-2.89	± 2.53	43.29	± 1.55
Camuesa fina d'Aragón	3372 AD	34.80	± 4.09	8.69	± 1.23	65.20	± 1.12	-0.89	± 6.49	38.11	± 0.87	43.29	± 0.77	117.61	± 0.75	65.29	± 1.65	-0.68	± 5.54	37.53	± 1.47
Cella	2512 AD	28.27	± 3.28	7.39	± 1.26	77.04	± 1.21	0.76	± 2.53	41.75	± 1.27	43.68	± 0.98	106.08	± 2.56	77.07	± 1.22	-0.04	± 2.71	42.29	± 1.39
Ciri Blanc	3402 AD	25.66	± 2.42	6.02	± 0.73	68.04	± 0.79	7.00	± 0.74	37.28	± 1.47	41.49	± 1.16	114.79	± 1.43	68.12	± 1.19	6.63	± 1.07	38.09	± 1.63
Cirio	3615 AD	23.62	± 3.02	5.07	± 0.66	68.87	± 2.44	-1.34	± 3.33	42.40	± 2.96	43.96	± 2.97	103.74	± 3.31	71.49	± 1.65	0.15	± 3.29	42.18	± 2.72
Cul de Cirio	3551 AD	24.18	± 1.78	4.67	± 0.44	70.42	± 1.55	3.32	± 4.20	46.85	± 2.75	49.85	± 2.11	109.46	± 3.06	72.59	± 1.49	-0.16	± 2.63	47.10	± 3.27
De Agosto	3619 AD	23.37	± 2.31	5.02	± 0.67	68.71	± 1.17	4.46	± 2.63	42.00	± 1.68	45.03	± 1.60	109.72	± 2.17	62.36	± 2.43	2.01	± 3.45	34.94	± 3.18
De pera	3416 AD	29.08	± 1.45	7.93	± 0.84	73.36	± 0.70	-0.95	± 2.29	44.41	± 1.19	45.41	± 0.75	101.09	± 3.27	72.85	± 0.89	-3.65	± 2.22	44.35	± 1.30
Del ciri	3413 AD	21.39	± 1.72	6.16	± 1.38	69.02	± 0.81	6.37	± 2.79	37.51	± 2.03	41.66	± 1.63	115.39	± 1.72	69.70	± 0.74	4.69	± 2.75	37.47	± 2.00
Esperiega	3420 AD	31.92	± 1.91	7.42	± 0.69	75.84	± 1.30	-0.72	± 3.55	44.88	± 1.72	46.44	± 0.99	102.78	± 3.64	75.64	± 1.14	-0.97	± 3.99	44.05	± 1.28
Eugenia	3468 AD	24.56	± 1.55	5.40	± 0.50	70.10	± 1.76	-10.13	± 6.98	46.99	± 3.21	47.42	± 2.05	109.37	± 4.96	65.49	± 2.53	-0.65	± 3.15	46.44	± 3.67
Guillemes	3411 AD	27.43	± 1.75	7.48	± 0.58	71.33	± 1.79	3.57	± 2.37	38.19	± 1.43	40.14	± 1.57	103.65	± 5.94	55.24	± 2.61	22.68	± 3.94	25.14	± 1.67
Helada	3368 AD	25.74	± 2.56	7.22	± 0.86	72.95	± 0.82	7.17	± 0.59	40.95	± 0.82	37.94	± 5.63	98.49	± 12.76	73.04	± 0.67	13.33	± 7.73	55.81	± 13.11
Hierro	3374 AD	33.33	± 1.47	8.71	± 0.63	72.11	± 1.03	6.33	± 2.82	46.16	± 1.14	49.43	± 1.01	111.09	± 0.69	60.55	± 5.04	12.80	± 5.45	34.89	± 4.00
Irgo-2	3622 AD	22.66	± 1.57	4.78	± 0.40	70.63	± 0.99	6.11	± 1.05	45.24	± 1.33	48.11	± 1.18	109.65	± 1.44	70.50	± 1.41	-1.93	± 2.15	46.26	± 2.72
Landetxo	3343 AD	27.94	± 1.96	6.75	± 1.19	67.24	± 2.54	-0.54	± 5.26	35.07	± 3.18	38.61	± 2.37	96.86	± 10.18	58.31	± 6.76	10.69	± 8.07	29.73	± 2.32
Les-1	3624 AD	28.56	± 1.59	5.42	± 0.52	69.88	± 0.89	-5.78	± 8.67	43.38	± 1.09	47.65	± 0.96	114.52	± 0.99	72.99	± 1.62	1.02	± 3.28	43.98	± 1.54
Les-2	3625 AD	33.28	± 1.53	6.16	± 0.92	68.87	± 1.45	4.09	± 3.84	43.27	± 1.82	46.39	± 1.52	110.44	± 2.83	68.98	± 1.22	-5.79	± 5.05	43.50	± 1.74
Mañaga	3554 AD	24.73	± 2.60	5.49	± 1.02	70.15	± 1.49	7.79	± 0.64	43.60	± 1.18	47.17	± 1.03	112.42	± 0.99	68.49	± 1.93	3.82	± 3.21	41.77	± 2.74
Mañaga	469 AD	26.74	± 1.96	5.69	± 0.84	69.56	± 1.07	2.76	± 4.55	43.79	± 1.55	40.13	± 6.52	99.02	± 11.94	69.59	± 1.87	10.87	± 9.76	56.49	± 12.62
Marinera	3412 AD	26.43	± 1.59	7.55	± 0.81	73.33	± 0.97	1.55	± 3.32	42.81	± 0.53	46.09	± 0.60	110.87	± 1.25	72.56	± 1.68	3.51	± 2.32	40.93	± 1.02
Marquinez	3419 AD	29.79	± 2.55	7.83	± 2.02	68.13	± 0.75	-4.10	± 6.60	38.87	± 0.94	43.78	± 0.91	116.67	± 0.84	67.39	± 1.03	-0.81	± 4.83	38.03	± 1.03
Montcada-1	3631 AD	23.04	± 2.23	5.29	± 0.51	70.78	± 1.99	5.96	± 2.62	45.17	± 2.04	48.19	± 1.96	107.46	± 4.81	61.48	± 2.54	10.36	± 3.99	36.85	± 3.31
Morro de Liebre	3256 AD	32.39	± 2.14	8.25	± 1.09	74.77	± 0.70	-0.43	± 4.27	39.85	± 1.35	42.59	± 1.52	111.44	± 1.38	69.40	± 1.69	1.81	± 2.93	37.31	± 1.80
Navalmoral de Bejar-1	3548 AD	30.34	± 2.47	5.97	± 0.87	69.70	± 1.11	-6.22	± 5.65	39.22	± 1.14	42.66	± 1.64	111.19	± 3.79	59.44	± 3.08	9.99	± 4.91	32.22	± 3.11
Nesple	3410 AD	37.12	± 2.32	8.49	± 1.09	66.33	± 3.02	6.68	± 5.70	32.46	± 1.86	32.11	± 3.03	70.12	± 20.58	56.58	± 3.17	26.14	± 5.87	35.64	± 12.63
Normanda	3252 AD	28.13	± 3.12	7.69	± 0.79	70.47	± 0.88	6.22	± 0.73	39.99	± 2.33	43.25	± 1.79	112.40	± 1.49	64.73	± 2.31	1.84	± 2.87	35.17	± 2.32
Ortell	413 AD	26.56	± 1.73	7.18	± 1.28	71.98	± 1.00	6.67	± 2.61	41.51	± 0.52	45.83	± 0.91	112.94	± 1.06	68.89	± 1.74	4.42	± 2.16	40.57	± 1.50

Online Resource 4 Continued

Accessions	Accession number	WE		DE		L (shaded)		a (shaded)		b (shaded)		C (shaded)		h (shaded)		L (blushed)		a (blushed)		b (blushed)	
Ortell	3546 AD	27.85	± 2.44	8.22	± 0.99	72.14	± 1.51	6.08	± 0.52	42.79	± 1.61	45.75	± 1.56	110.81	± 1.03	67.57	± 2.00	-0.08	± 2.58	40.24	± 2.50
Pera 2	3417 AD	27.04	± 1.74	7.62	± 0.88	73.05	± 0.72	-4.38	± 2.29	44.27	± 0.96	45.12	± 0.79	100.19	± 2.22	71.93	± 1.18	-3.45	± 2.17	45.52	± 1.16
Pera de Sangüesa	3379 AD	28.93	± 2.32	7.09	± 0.97	66.79	± 1.24	4.71	± 0.93	45.44	± 1.51	47.96	± 1.14	108.12	± 1.45	65.97	± 1.76	0.82	± 2.47	45.26	± 1.79
Pero pardo	3369 AD	30.57	± 2.09	8.10	± 0.91	68.09	± 0.90	1.66	± 2.19	47.27	± 1.75	48.41	± 1.34	104.47	± 2.55	68.34	± 1.40	2.01	± 1.78	47.60	± 2.33
Peromingán	1158 AD	20.52	± 1.76	5.97	± 0.75	71.81	± 0.96	1.91	± 4.89	36.82	± 0.82	40.60	± 0.79	115.52	± 1.47	72.47	± 1.21	1.13	± 4.86	37.07	± 0.94
Peruco de Caparroso	3373 AD	29.02	± 2.41	8.14	± 1.93	64.19	± 0.87	-4.38	± 5.78	38.99	± 1.56	43.24	± 1.39	115.21	± 2.28	59.36	± 1.99	2.26	± 5.39	33.19	± 2.24
Poma de San Juan	3556 AD	22.00	± 2.35	5.98	± 0.58	76.82	± 1.08	2.47	± 3.26	41.78	± 1.00	44.37	± 1.26	109.20	± 2.02	67.95	± 4.45	6.34	± 5.39	36.23	± 3.83
Prau Riu 3	3491 AD	25.91	± 1.58	6.06	± 0.99	54.44	± 8.25	9.17	± 7.02	28.47	± 4.65	33.23	± 4.58	79.51	± 20.19	43.49	± 6.76	18.59	± 6.62	18.53	± 3.63
Prau Riu 4	3492 AD	33.90	± 2.43	3.91	± 1.02	71.39	± 1.17	4.26	± 2.49	44.61	± 1.74	47.25	± 1.65	108.28	± 2.62	67.20	± 1.74	0.99	± 3.13	40.45	± 2.87
Prau Riu 5	3493 AD	29.03	± 2.11	7.61	± 0.72	61.28	± 1.46	-3.18	± 1.78	37.15	± 1.19	37.72	± 1.24	97.13	± 2.76	63.22	± 1.49	-0.90	± 2.15	38.44	± 1.91
Rebellón	3370 AD	26.59	± 1.73	6.09	± 0.71	68.89	± 0.98	-1.69	± 4.65	41.21	± 0.78	44.42	± 1.14	109.81	± 2.88	60.04	± 2.49	3.31	± 5.36	33.51	± 2.38
Reguard-1	3616 AD	22.44	± 1.84	5.55	± 0.80	69.39	± 1.74	6.61	± 2.00	42.83	± 2.13	46.24	± 1.77	111.81	± 2.34	69.15	± 1.40	0.74	± 4.22	44.31	± 3.54
Reineta Blanca Canadá	308 AD	34.20	± 2.48	6.85	± 0.76	67.00	± 1.35	-5.49	± 1.48	44.62	± 1.47	45.22	± 1.15	99.15	± 1.31	65.99	± 1.82	-3.20	± 2.23	46.24	± 1.47
Reineta Blanca Canadá	3194 AD	32.43	± 1.66	6.75	± 0.83	67.34	± 1.32	-5.10	± 2.15	46.14	± 1.64	46.90	± 1.24	99.17	± 2.05	65.16	± 1.79	-3.68	± 2.08	46.15	± 1.77
Reineta Inesita Asua	2543 AD	30.29	± 1.66	6.37	± 0.87	69.65	± 0.91	-4.73	± 10.89	41.50	± 0.91	45.38	± 0.91	115.40	± 1.49	64.03	± 3.95	-1.53	± 10.36	34.33	± 4.26
Reineta Regil	3466 AD	27.75	± 1.69	6.65	± 0.94	62.68	± 6.30	4.48	± 1.47	37.12	± 5.35	41.03	± 4.25	104.71	± 14.74	61.58	± 8.65	2.33	± 2.79	37.14	± 5.38
Reneta	3408 AD	33.67	± 1.16	7.40	± 0.76	65.61	± 2.32	6.63	± 4.39	36.54	± 2.71	38.53	± 2.52	79.60	± 16.87	49.33	± 3.41	28.67	± 3.81	34.31	± 11.08
Roja Valle Benejama	1038 AD	30.50	± 1.75	7.24	± 0.75	69.54	± 2.01	1.08	± 3.25	37.32	± 1.87	38.88	± 2.11	104.11	± 4.94	45.88	± 3.27	27.76	± 5.19	23.13	± 2.31
Roser de la Reula	3552 AD	24.69	± 1.68	4.14	± 0.56	70.41	± 2.00	5.54	± 3.15	40.10	± 2.20	42.75	± 1.94	100.74	± 9.51	56.92	± 2.11	16.14	± 4.58	29.45	± 2.42
Ruixou-1	3614 AD	24.09	± 2.12	5.55	± 0.56	67.80	± 1.73	4.54	± 2.41	47.72	± 3.74	50.13	± 3.06	108.04	± 3.12	65.86	± 2.19	1.58	± 3.62	47.46	± 4.72
San Miguel	2579 AD	32.40	± 1.68	5.99	± 0.54	59.12	± 6.28	5.64	± 5.83	31.28	± 4.87	34.22	± 3.69	79.43	± 16.24	44.70	± 4.68	26.32	± 4.95	19.30	± 3.05
Sandía	3336 AD	29.93	± 2.68	8.75	± 1.42	65.57	± 3.27	-6.14	± 7.50	38.69	± 2.80	40.99	± 3.10	104.55	± 9.31	56.08	± 5.08	10.69	± 8.11	30.64	± 4.28
Sant Joan	3409 AD	25.92	± 3.82	4.51	± 0.84	70.26	± 1.09	-2.29	± 8.44	38.57	± 1.16	43.85	± 1.07	117.01	± 0.67	69.92	± 2.20	1.80	± 4.69	35.73	± 2.85
Santa Margarida	3401 AD	26.24	± 2.02	6.67	± 1.23	69.25	± 4.34	-4.55	± 6.20	39.64	± 3.32	43.88	± 3.17	108.60	± 9.11	64.29	± 4.57	0.97	± 5.69	34.67	± 3.53
Signatillis	3403 AD	39.12	± 2.81	10.29	± 1.67	70.95	± 0.87	-3.96	± 6.85	41.60	± 0.61	46.25	± 0.84	115.06	± 1.44	71.72	± 1.63	-1.13	± 4.89	41.80	± 1.09
Solafuente	3559 AD	29.86	± 1.70	5.98	± 0.93	65.68	± 1.59	3.24	± 2.81	36.89	± 2.77	39.24	± 3.37	107.57	± 4.24	53.48	± 3.59	12.75	± 5.48	26.35	± 3.70
Taüll-1	3623 AD	27.20	± 2.80	6.84	± 1.62	69.73	± 1.06	4.37	± 1.16	39.18	± 1.87	41.86	± 1.83	110.23	± 1.79	69.39	± 1.01	0.92	± 2.75	41.65	± 2.42
Tempera	3334 AD	29.85	± 2.36	7.45	± 0.89	69.96	± 0.96	4.85	± 4.41	37.91	± 1.06	42.10	± 0.95	116.36	± 0.60	69.90	± 1.23	1.69	± 5.08	39.02	± 1.53
Terrera	3469 AD	34.23	± 3.80	8.08	± 0.73	51.22	± 1.01	6.38	± 1.32	33.66	± 1.16	34.45	± 1.13	79.12	± 2.22	50.96	± 2.00	7.20	± 1.15	33.22	± 1.20
Toxta	3471 AD	30.60	± 1.70	5.86	± 0.77	70.19	± 0.91	2.00	± 4.39	39.78	± 1.25	43.21	± 1.09	112.39	± 2.33	68.93	± 1.12	-0.54	± 2.42	40.97	± 1.95
Transparente	3377 AD	33.67	± 1.84	8.68	± 1.82	68.74	± 0.92	-8.48	± 6.78	40.58	± 1.13	45.08	± 1.09	116.68	± 0.53	68.30	± 2.12	-0.81	± 5.72	40.33	± 1.97
Transparente Blanca	3344 AD	30.27	± 2.16	5.80	± 0.54	75.23	± 1.44	-3.44	± 5.94	40.90	± 1.26	44.78	± 1.45	113.06	± 1.70	75.44	± 1.67	-1.11	± 5.38	41.70	± 1.58
Urarte	3415 AD	31.69	± 2.68	8.10	± 1.75	66.02	± 1.15	-5.47	± 6.79	39.34	± 1.07	43.95	± 0.98	116.94	± 0.64	67.20	± 1.38	-3.78	± 6.45	39.43	± 1.13
Urtebete	3345 AD	35.53	± 1.89	8.46	± 0.79	70.98	± 0.68	0.94	± 4.88	42.84	± 0.75	46.23	± 0.95	110.98	± 2.02	71.64	± 0.84	3.79	± 3.39	43.43	± 0.98
Valsaina	3558 AD	32.16	± 1.57	7.67	± 1.19	69.14	± 1.41	-2.94	± 5.28	50.46	± 2.17	52.83	± 1.76	97.87	± 6.01	60.21	± 1.55	13.16	± 4.88	42.73	± 2.48
Verde Doncella	310 AD	32.64	± 2.84	9.09	± 0.96	77.18	± 1.03	5.19	± 1.15	42.01	± 0.93	44.82	± 1.07	109.69	± 1.38	78.71	± 1.21	-4.06	± 1.84	43.03	± 1.49
Vinçada Tardia	3621 AD	37.00	± 1.68	8.00	± 0.91	68.83	± 1.63	0.93	± 2.62	43.17	± 2.30	45.06	± 2.16	105.63	± 2.51	69.97	± 1.52	-1.43	± 2.72	44.69	± 2.31

WE: width of eye basin; DE: depth of eye basin;

Online Resource 5 Apple accessions maintained at the Experimental Station of Aula Dei (CSIC-Zaragoza, Spain). Collection information includes name and bank number and mean values of each trait evaluated in this study.

Accessions	Accession number	C (blushed)		h (blushed)		FF		SSC		TA		RI	
Acipres	3339 AD	34.45	± 1.37	53.69	± 9.63	85.60	± 0.73	14.21	± 0.45	2.37	± 0.21	6.36	± 0.78
Almenar-2	3555 AD	59.86	± 14.40	71.69	± 11.68	71.16	± 0.94	14.48	± 1.07	2.21	± 0.33	7.53	± 1.46
Ascara 1	3423 AD	36.37	± 2.25	76.89	± 15.97	79.30	± 0.75	11.37	± 0.52	10.28	± 0.75	1.19	± 0.14
Ascara 2	3424 AD	34.75	± 1.84	71.98	± 12.64	81.14	± 0.64	12.16	± 0.49	10.56	± 0.61	1.20	± 0.09
Astrakán roja	3378 AD	36.49	± 1.92	90.53	± 11.42	53.13	± 0.70	12.54	± 0.75	15.65	± 1.31	0.89	± 0.07
Audiena de Oroz	3375 AD	38.04	± 0.85	114.65	± 1.83	80.41	± 0.64	10.51	± 0.76	6.72	± 0.97	1.89	± 0.30
Bellaguardia Lardero	3547 AD	43.50	± 1.77	102.39	± 2.28	49.16	± 0.89	12.35	± 0.42	11.01	± 1.25	1.18	± 0.12
Boluaga	3340 AD	32.41	± 2.28	81.05	± 12.06	85.40	± 0.85	13.09	± 0.90	7.63	± 0.97	1.79	± 0.21
Bossost-1	3626 AD	38.82	± 1.69	86.88	± 8.59	68.82	± 0.72	13.15	± 0.72	13.07	± 1.88	1.10	± 0.16
Bossost-4	3629 AD	42.65	± 3.37	93.71	± 7.95	95.24	± 0.77	14.57	± 1.06	8.13	± 0.43	1.74	± 0.16
Bossost-5	3630 AD	39.31	± 4.05	91.97	± 5.67	74.93	± 0.97	13.00	± 0.94	11.48	± 1.71	1.22	± 0.18
Bost Kantoia	3341 AD	46.79	± 0.99	106.47	± 3.00	78.60	± 0.56	14.05	± 0.65	7.05	± 0.56	2.08	± 0.21
Cabdellá-2	3613 AD	36.73	± 2.12	38.64	± 8.60	80.18	± 0.43	14.64	± 1.00	12.53	± 1.23	1.29	± 0.23
Calvilla de San Salvador	3342 AD	39.90	± 3.21	105.22	± 9.09	73.68	± 0.59	10.03	± 0.42	14.77	± 1.32	0.69	± 0.07
Camosa	3620 AD	41.55	± 3.26	86.19	± 7.24	68.69	± 0.61	14.67	± 0.82	5.37	± 0.70	2.91	± 0.60
Camuesa del Llobregat	1342 AD	44.18	± 1.46	99.90	± 3.01	57.67	± 0.57	14.62	± 1.38	8.38	± 3.72	3.96	± 1.27
Camuesa fina d'Aragón	3372 AD	41.60	± 1.45	115.09	± 2.77	73.68	± 0.45	10.20	± 0.71	17.34	± 1.12	0.58	± 0.05
Cella	2512 AD	44.15	± 1.29	104.76	± 3.28	52.88	± 0.63	14.23	± 0.66	2.39	± 0.20	6.39	± 0.80
Ciri Blanc	3402 AD	41.66	± 1.25	114.26	± 1.94	67.05	± 0.38	12.03	± 1.18	1.68	± 0.21	7.88	± 1.46
Cirio	3615 AD	43.52	± 2.24	100.66	± 5.89	73.63	± 0.89	16.83	± 2.02	7.62	± 0.97	2.30	± 0.53
Cul de Cirio	3551 AD	48.45	± 3.14	101.37	± 4.17	66.79	± 0.28	13.62	± 0.94	1.68	± 0.23	8.68	± 1.47
De Agosto	3619 AD	35.93	± 3.20	89.44	± 7.26	59.88	± 0.77	12.63	± 0.96	6.26	± 0.50	2.03	± 0.20
De pera	3416 AD	45.30	± 1.06	101.49	± 3.06	79.03	± 0.52	15.31	± 0.84	8.85	± 0.82	1.72	± 0.14
Del ciri	3413 AD	40.84	± 2.15	111.62	± 5.08	72.54	± 0.63	13.10	± 1.16	1.74	± 0.31	7.70	± 0.85
Esperiega	3420 AD	45.53	± 1.02	105.08	± 3.15	67.86	± 0.60	13.65	± 0.66	8.36	± 0.39	1.68	± 0.11
Eugenia	3468 AD	43.29	± 3.30	92.62	± 5.35	112.42	± 0.48	15.23	± 1.02	8.14	± 0.76	1.98	± 0.25
Guillemes	3411 AD	34.74	± 1.46	51.93	± 7.93	74.35	± 0.62	12.55	± 0.70	6.70	± 0.69	2.01	± 0.19
Helada	3368 AD	44.60	± 0.89	111.20	± 2.00	80.58	± 0.59	13.37	± 0.64	3.75	± 0.38	3.60	± 0.30
Hierro	3374 AD	39.60	± 3.41	76.73	± 11.88	90.64	± 0.56	13.28	± 0.26	8.93	± 0.43	1.49	± 0.09
Irgo-2	3622 AD	46.96	± 2.70	98.04	± 3.72	76.52	± 0.52	13.36	± 0.35	5.05	± 0.78	2.82	± 0.44
Landetxo	3343 AD	34.87	± 4.11	80.23	± 14.06	47.49	± 0.62	13.34	± 0.68	15.13	± 1.04	0.90	± 0.06
Les-1	3624 AD	45.76	± 1.77	103.50	± 5.04	65.73	± 0.56	11.55	± 0.64	3.67	± 0.26	3.13	± 0.22
Les-2	3625 AD	45.70	± 1.55	105.11	± 5.28	76.41	± 0.84	11.35	± 0.60	10.33	± 1.05	1.14	± 0.11
Mañaga	3554 AD	44.20	± 2.35	101.10	± 7.80	80.63	± 0.52	12.88	± 0.49	4.89	± 0.41	2.60	± 0.23
Mañaga	469 AD	46.52	± 1.83	109.01	± 4.57	70.30	± 0.61	11.11	± 0.66	3.75	± 0.48	3.20	± 0.39
Marinera	3412 AD	43.14	± 1.06	104.17	± 4.48	48.81	± 0.39	11.22	± 0.32	12.29	± 1.22	0.97	± 0.09
Marquinez	3419 AD	41.17	± 1.41	111.78	± 3.46	66.25	± 0.54	10.66	± 0.48	13.81	± 0.83	0.76	± 0.07
Montcada-1	3631 AD	39.97	± 2.59	77.35	± 8.13	77.26	± 0.53	14.84	± 0.57	4.79	± 0.42	3.31	± 0.37
Morro de Liebre	3256 AD	38.36	± 1.62	95.67	± 5.04	75.23	± 0.40	12.08	± 0.59	3.82	± 0.31	3.36	± 0.41
Navalmoral de Bejar-1	3548 AD	36.37	± 2.60	80.26	± 11.53	78.37	± 0.39	12.48	± 1.04	8.52	± 1.24	1.72	± 0.39
Nesple	3410 AD	33.39	± 2.68	48.81	± 9.16	61.75	± 0.74	12.68	± 0.71	5.61	± 0.54	2.37	± 0.16
Normanda	3252 AD	36.20	± 1.96	94.82	± 7.23	93.71	± 0.66	12.10	± 0.47	5.59	± 0.27	2.19	± 0.17
Ortell	413 AD	42.99	± 1.99	106.86	± 4.93	84.02	± 0.32	14.25	± 0.75	3.52	± 0.24	4.15	± 0.32

Online Resource 5 Continued

Accessions	Accession number	C (blushed)			h (blushed)			FF		SSC		TA		RI					
Ortell	3546 AD	41.22	±	2.49	97.08	±	5.69	78.61	±	0.65	12.44	±	0.56	3.88	±	0.27	3.37	±	0.26
Pera 2	3417 AD	45.96	±	1.09	96.62	±	3.26	74.95	±	0.54	16.17	±	0.58	10.12	±	1.22	1.65	±	0.19
Pera de Sangüesa	3379 AD	47.12	±	1.58	105.34	±	2.80	68.26	±	0.48	15.30	±	0.67	7.31	±	0.57	2.09	±	0.19
Pero pardo	3369 AD	48.86	±	2.12	99.01	±	4.48	71.03	±	0.66	16.21	±	0.40	6.44	±	0.46	2.52	±	0.19
Peromingán	1158 AD	40.88	±	0.80	114.86	±	2.07	71.82	±	0.82	10.63	±	0.67	12.55	±	0.85	0.89	±	0.10
Peruco de Caparroso	3373 AD	36.77	±	2.04	99.10	±	10.07	62.60	±	0.55	11.82	±	0.61	2.76	±	0.11	4.43	±	0.24
Poma de San Juan	3556 AD	39.80	±	2.10	81.82	±	10.12	63.40	±	0.66	11.22	±	0.41	3.20	±	0.35	3.68	±	0.34
Prau Riu 3	3491 AD	28.87	±	4.13	50.24	±	17.78	93.64	±	1.09	13.17	±	0.58	6.11	±	0.37	2.19	±	0.13
Prau Riu 4	3492 AD	41.66	±	2.70	91.65	±	6.06	68.79	±	0.84	13.20	±	0.73	11.73	±	1.51	1.23	±	0.16
Prau Riu 5	3493 AD	39.42	±	2.00	98.37	±	4.01	79.58	±	0.56	13.52	±	0.89	13.23	±	2.15	1.26	±	0.39
Rebellón	3370 AD	36.45	±	2.05	87.44	±	9.18	74.17	±	0.60	11.94	±	0.47	8.96	±	0.63	1.36	±	0.12
Reguard-1	3616 AD	46.01	±	3.25	100.09	±	7.11	83.75	±	0.69	14.09	±	0.90	4.35	±	0.38	3.42	±	0.41
Reineta Blanca Canadá	308 AD	47.26	±	1.19	98.68	±	3.30	67.62	±	0.68	16.52	±	0.97	11.11	±	1.12	1.51	±	0.12
Reineta Blanca Canadá	3194 AD	46.73	±	1.67	97.89	±	2.89	61.79	±	0.66	15.99	±	0.93	9.99	±	0.60	1.62	±	0.09
Reineta Inesita Asua	2543 AD	37.70	±	3.26	95.85	±	14.05	64.93	±	0.49	10.74	±	0.53	12.36	±	1.42	0.90	±	0.12
Reineta Regil	3466 AD	39.44	±	5.61	104.47	±	13.77	100.05	±	0.58	16.59	±	1.36	7.57	±	0.84	2.25	±	0.23
Reneta	3408 AD	36.17	±	1.16	43.14	±	8.11	77.30	±	0.53	12.54	±	0.39	3.65	±	0.34	3.51	±	0.26
Roja Valle Benejama	1038 AD	37.27	±	1.55	43.79	±	9.71	73.49	±	0.79	11.81	±	0.30	3.55	±	0.43	3.43	±	0.34
Roser de la Reula	3552 AD	34.60	±	2.08	63.61	±	8.96	89.97	±	0.69	13.51	±	0.34	5.98	±	0.62	2.36	±	0.28
Ruixou-1	3614 AD	48.37	±	4.51	92.58	±	6.33	63.17	±	1.31	14.74	±	1.38	2.91	±	0.26	5.66	±	0.57
San Miguel	2579 AD	32.08	±	3.83	37.76	±	7.92	75.69	±	0.92	13.92	±	0.35	4.11	±	0.63	3.63	±	0.74
Sandía	3336 AD	38.45	±	2.37	77.34	±	16.84	59.23	±	0.74	12.06	±	0.54	9.29	±	0.77	1.33	±	0.12
Sant Joan	3409 AD	38.62	±	2.06	102.65	±	7.70	71.62	±	0.66	11.12	±	0.50	6.15	±	0.56	1.87	±	0.21
Santa Margarida	3401 AD	37.66	±	3.12	94.89	±	11.77	56.64	±	0.92	11.10	±	0.44	16.03	±	1.53	0.71	±	0.08
Signatillis	3403 AD	45.02	±	1.30	110.28	±	3.36	71.85	±	0.35	11.55	±	0.36	10.06	±	0.85	1.16	±	0.11
Solafuente	3559 AD	32.20	±	2.41	66.67	±	12.01	70.24	±	0.49	11.20	±	0.52	8.02	±	0.81	1.44	±	0.13
Taüll-1	3623 AD	43.34	±	1.81	105.01	±	4.88	94.23	±	0.55	13.50	±	0.55	7.78	±	0.76	1.77	±	0.21
Tempera	3334 AD	43.04	±	1.31	114.39	±	2.78	81.34	±	0.71	12.13	±	0.50	8.07	±	0.62	1.49	±	0.09
Terrera	3469 AD	34.15	±	1.12	78.38	±	2.20	69.90	±	0.90	18.09	±	1.47	13.50	±	1.93	1.37	±	0.13
Toxta	3471 AD	42.36	±	1.87	103.52	±	4.35	70.52	±	0.83	17.22	±	0.62	7.04	±	0.50	2.53	±	0.26
Transparente	3377 AD	44.13	±	1.89	110.47	±	5.66	70.93	±	0.66	11.42	±	0.60	12.74	±	0.75	0.94	±	0.07
Transparente Blanca	3344 AD	45.08	±	1.98	111.69	±	2.41	64.44	±	0.62	11.05	±	0.46	13.38	±	0.79	0.82	±	0.06
Urarte	3415 AD	43.80	±	1.40	114.97	±	3.08	66.66	±	0.60	10.16	±	0.47	15.72	±	2.25	0.90	±	0.36
Urtebete	3345 AD	46.40	±	0.93	110.98	±	1.54	82.46	±	0.50	11.96	±	0.30	8.35	±	0.94	1.52	±	0.16
Valsaina	3558 AD	46.42	±	2.71	77.20	±	7.36	75.07	±	0.51	14.54	±	0.84	8.98	±	1.12	1.75	±	0.30
Verde Doncella	310 AD	44.04	±	1.59	101.06	±	2.02	75.15	±	0.68	13.40	±	0.33	1.85	±	0.19	7.45	±	0.79
Vinçada Tardia	3621 AD	45.93	±	2.01	101.15	±	4.01	70.60	±	1.16	14.41	±	0.65	10.63	±	1.41	1.43	±	0.20

FF: flesh firmness; SSC: soluble solids content; TA: titratable acidity; RI: ripening index.