Genetic origin and climate determine fruit quality and antioxidant traits on apple (*Malus x domestica* Borkh)

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Abstract

Apples are highly appreciated in terms of flavor, nutritional and health-promoting compounds and are associated with a reduced risk to develop age-related and chronic diseases. In this study, 155 accessions of *Malus x domestica* Borkh from the apple germplasm collection, situated at the Experimental Station of Aula Dei in NE Spain, were analyzed during five consecutive years (2014-2018). Basic fruit quality traits including soluble solids content (SSC), titrable acidity (TA) and the ratio SSC/TA (ripening index - RI) were obtained. In addition, biochemical compounds such as total phenolics content (TPC), flavonoids (TFC), vitamin C (Ascorbic acid - AsA) and relative antioxidant capacity (RAC) were determined. Statistical analysis was used to determine differences in trait values among accessions and years. The well adapted local accessions showed, in general, higher average content of antioxidants and RAC compared with the foreign and commercial ones. A multivariate model was fitted with the accessions and climate features of each year as independent variables. A cluster analysis was then performed on the model coefficients space to classify the 155 accessions within five groups. The cluster analysis showed that foreign cultivars (i.e., those not originating from Spain) were concentrated in two groups while local accessions could not be segregated and had very different profiles. Furthermore, the concentration of bioactive compounds tended to decrease with higher temperatures, while increased with higher solar radiation. Statistical analyses emphasized differences between groups and highlighted accessions and climate as main factors affecting metabolite profiles and fruit characteristics.

Keywords: Flavonoids, meteorogical parameters, mixed-effects model, phenols, plant breeding, vitamin C

1. Introduction

Apple (*Malus x domestica* Borkh, family *Rosaceae*, tribe *Pyreae*) is among the most widely consumed fresh fruits in the world. It has a special importance as one of the major temperate fruit crops cultivated globally. In 2019, more than 87 M tonnes were produced worldwide (FAOSTAT, 2021) and it ranked second in production after peaches and nectarines, and before pears, in Spain with more than 638 thousands of tonnes. Since its consumption is widespread in many countries and it is available on the market for the whole year, apple is, among fruits and vegetables, a major source of nutrients and bioactive compounds for humans (Michalska & Łysiak, 2015). The content of antioxidants present in apples is also important because of their contribution to the sensory quality of fresh fruit and processed apple products (Khanizadeh et al., 2008). Bioactive compounds improve the quality and the shelf life of vegetables and reduce the risk of post-harvest diseases (Bui et al., 2019; Davey et al., 2007). They also provide important health benefits to humans (Boeing et al., 2012; Gibney et al., 2019; Ho et al., 2020). In fact, apples are an important dietary source of potentially healthy biomolecules such as antioxidants. Moreover, there is an increasing evidence of a relationship between the consumption of fruits and vegetables and a reduced risk of human diseases such as cancer, heart coronary, cardiovascular, diabetes, Alzheimer’s diseases, and age-related functional decline (Zhang et al., 2016).

Currently, there are more than 7,000 documented apple cultivars in the world (Urrestarazu et al., 2016; Pereira-Lorenzo et al., 2017). However, the global production is dominated by relatively few well-adapted cultivars (Fuji, Gala, Golden, Granny Smith and Delicious), many of which are closely related (Urrestarazu et al., 2016; Ordidge et al., 2018), to the detriment of the locally well-adapted apple cultivars (Reig et al., 2015). This fact is leading to a dramatic loss of genetic diversity in the orchards and may also hamper future plant breeding while breeders require genetic variation for plant improvement (Swarup et al., 2020). Therefore, apple collections play a crucial role in preserving genetic diversity and providing breeding material (Muranty et al., 2020; Reig et al., 2015). Several apple germplasm banks are presently maintained in Spain, preserving mainly old cultivars which have been grown traditionally in their respective regions of origin (Pereira-Lorenzo et al., 2017), but also other foreign or commercial cultivars. Conservation of genetic diversity is important not only for wild species but also for cultivated plant species (Font i Forcada et al., 2014a; Guajardo et al., 2020; Reig et al., 2015). Monoculturalization has already driven out many local cultivars. Consequently, the collection, conservation and evaluation of local or old cultivars and wild relatives in germplasm banks are urgently required to prevent their extinction. Genetic diversity in local cultivars and wild relatives is thus very important, as it preserves genes that might be relevant from agronomical and fruit quality points of view (Reig et al., 2015; Font i Forcada et al., 2019a; Guajardo et al., 2020).

Preserving genetic variability is especially relevant in the context of climate change (Parajuli, 2019; Parry, 2019). Indeed, the complex quantitative nature of basic fruit quality traits and bioactive compounds can be affected by environmental conditions and agronomical management (Stewart & Ahmed, 2020). Actually, environmental variables such as climate parameters (solar radiation, precipitation, temperature …) influence the tree-growing environment providing wide variations in bioactive compounds accumulation (Cirilli et al., 2016; Yuri et al., 2009). The increasing temperatures observed in recent decades have already had a visible impact on plant growth and development (Fujisawa & Kobayashi, 2011; Li et al., 2020). Farmers and researchers are changing their behavior by boosting and selecting climate-resilient apple cultivars (Beguería et al., 2019; Boudichevskaia et al., 2020). At this respect, there is a need to improve our understanding on how climate affects relevant fruit traits, and germplasm banks offer a good platform to assess this concern (Swarup et al., 2020). Climate experts have anticipated an increase in air temperature in the range of 2–5 °C (Benlloch-González et al., 2018) in the Mediterranean Basin.

To the best of our knowledge, no study has previously focused on the study of antioxidant compounds of local apple cultivars grown in Spain and conserved in germplasm banks. Nevertheless, most local and traditionally grown Spanish accesions have been characterized with SSR markers (Pereira-Lorenzo et al., 2017) as well as using morphological and phenological parameters (Reig et al., 2015). In other countries, several studies have reported apple antioxidant compounds only for a few number of cultivars and one or two years (McClure et al., 2019; McGhie et al., 2005; Yuri et al., 2009), in comparison with the 155 accesions and the five years considered in the present work. Moreover, studies concerning climate relationship with fruit quality traits are really scarce (Ahmadi et al., 2019; Kim et al., 2019).

Therefore, the goal of this study is to better characterize a higher number of apple accessions, increase the knowledge of nutritional fruit quality in local accesions and commercial cultivars, and search for the role of the climate and its importance onto the apple fruit quality to facilitate breeders improving nutraceutical properties and facing the changes due to global warming. To do that, fruit quality and biochemical compounds of 155 accessions from the first germplasm bank established in Spain will be assessed. In addition, a mixed model will be built searching the relationship between climate parameters and fruit quality traits.

2. Materials and methods

2.1. Plant material and field trial

The research work was conducted on the apple germplasm bank established at the Experimental Station of Aula Dei (EEAD-CSIC, Zaragoza, NE Spain: 41° 43′ 42.7″ N, 0° 48′ 44.1″ W). A total of 155 apple accessions [*Malus x domestica* Borkh], consisting of 99 local accesions and 56 foreign accessions were studied (Table 1). Indeed, most of the foreign accessions are commercial cultivars meanwhile, within the local accessions, autochtone commercial cultivars and traditional landraces are represented. The accessions were classified according to their skin color as bicolor (95 accessions), red (9), green (34), yellow (14) and brown (3), with brown corresponding to skin completely covered by russeting. Each accession had three-tree replications established in a unique block design in the orchard. 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.

2.2. Leaf and Fruit sampling

To assess the ploidy level of the different accessions studied, newly expanded mature leaves were collected from each accession and analysed as described in Reig et al. (2015). The accessions were therefore classified into diploids (129 accessions) and triploids (26 accessions).

Regarding to the fruit sampling, a representative sample of 15 fruits (5 fruits × tree × rep.) were harvested when fruit firmness (FF) attained a value around 70–80 N or when they exhibited the ground color representative. Maturity date ranged from late June to early December, depending on the accession. Fruit traits were measured for each accession at least three years within the period 2014-2018, and means for each season and accession were calculated.

2.3. Basic fruit quality traits

Soluble solid content (SSC) and titratable acidity (TA) were determined on flesh juice extracted by an automatic juicer (Philips, HR185890) with three replicates per accession and five fruits per replicate. Soluble solid content of fruit juice was measured with a digital refractometer (Atago PR-101, Tokyo, Japan) and was expressed as ºBrix. Titratable acidity was determined using an automatic titration system (EasyPlus Titrator, Mettler Toledo, US) with 0.1 N NaOH to a pH end point of 8.1. Results were expressed as g malic acid per liter. Ripening index (RI) was calculated based on the SSC/TA ratio.

2.4. Phytochemical traits

For the analysis of the total phenolics content (TPC), total flavonoids content (TFC), vitamin C (ascorbic acid – AsA) and the relative antioxidant capacity (RAC), a flesh sample composite of 5 g of five peeled fruits per replicate was frozen in liquid nitrogen and kept at −20°C until further analysis. Three replicates per accession were sampled and prepared. Samples were homogenized in a polytron (T25D Ultra-Turrax, IKA Works Inc., Wilmington, NC, USA) after one night in 10 mL of extraction solution [methanol/Milli-Q water, 80% (v/v) for TPC, TFC and RAC, and metaphosphoric acid, 5% (w/v) for vitamin C - AsA]. Extracts were centrifuged at

20,000 g for 30 min at 4°C, and the supernatant was collected and stored at −20°C. The phytochemical compounds were analyzed using a 96-well microplate spectrophotometer photodiode array detector (Asys UVM 340 microplate reader; Biochrom, Cambridge, UK) as described by Font i Forcada et al. (2019a). The standard calibration curves were daily prepared on each microplate and eight different concentrations were used.

TPC was determined using the Folin-Ciocalteau method (Singleton et al., 1965) with modifications. Ninety microliters of diluted extract (1:8) were mixed with 80 µL of Folin-Ciocalteau reagent (0.25 N). The sample was incubated for three minutes before adding 30 µL of sodium carbonate [NaCO3, 11% (w/v)]. Then it was incubated for one hour in the dark at room temperature. Absorbance was measured at 725 nm and the results expressed in mg of GAE per 100 g of FW.

TFC was determined using a colorimetric assay based on the method of Zhishen et al. (1999) with minor modifications. Fifty microliters of the extract were mixed with 100 µL of Milli-Q water, 15 µL of sodium nitrite [$NaNO_2$, 5% (w/v)] and incubated for five minutes before adding 15 µL of aluminium chloride [$AlCl_3$, 10% (w/v)]. The microplate was incubated for three minutes more and finally 100 µL of sodium hydroxide 4% (NaOH) was added. Absorbance was measured at 510 nm. The results were expressed in mg CE per 100 g FW.

The RAC was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method adapted from Brand-Williams et al. (1995) with modifications. Ten microliters of the extract were mixed with 290 µL of DPPH (100µM in methanol, 80:20, v/v) and incubated in the dark at room temperature for 10 min. The absorbance was measured at 515 nm. The results were expressed in mg Trolox per 100 g FW.

Vitamin C – AsA was determined using the method for the spectrophotometric determination of ascorbic acid (AsA) as described by Zaharieva and Abadía (2003) with modifications. The extract (75 µL) was mixed with 75 µL of Milli-Q water, 60 µL of phosphoric acid diluted (H_3PO_4 85%/Milli-Q water 1:1), 60 µL of 2,2-bipyridyl [2,2-bipyridyl, 40:60, (w/v)/ethanol, 70:30, (v/v)] and 30µL of iron (III) chloride $FeCl_3.6H_2O$, 3% (w/v). Then it was incubated in a MemmertTM oven during one hour at 37ºC. Absorbance was measured at 525 nm and the results were expressed in mg AsA per 100 g FW.

2.5. Climate data

Climate variables as relative humidity (rh), maximum, minimum and mean temperature (tmx, tmn and tmd), rainfall (pre), and solar radiation (rad), were recorded daily during the study period (1st January 2014 to 31st December 2018). Data were downloaded from the Aula Dei meteorological station, nearby the orchard, which belongs to the official network of the Spanish meteorological service (AEMET). Relative humidity, maximum and minimum temperatures were daily recorded on a Rotronic HC2-S3 thermometer. Mean temperature was calculated as the average between tmx and tmn. Precipitation was recorded using a Pulsos ARG100 automatic raingage. Finally, solar radiation was measured on a Skye SP1110 pyranometer. The data were recorded automatically in a data-logger, and downloaded daily to a central server.

2.6. Data analysis

All statistical analyses were carried out using R software (R Development Core Team, 2019). The one-way analysis of variance (ANOVA) was run to determine whether there were any statistically significant differences between the means of the evaluated traits for the five years of study. The year effect, the accession, and their interaction (accession x year) effect were considered. The Pearson's correlations and a Principal Component analysis (PCA) were performed to understand how biochemical traits contribute to variability among accessions. A linear mixed-effects model (Pinheiro et al., 2019) was constructed using the different traits studied as dependent variables, the climate features of each growing season as independent variables or fixed effects, and the cultivar as random effect affecting both the intercept and the fixed effects coefficients. The growing season for the mixed-effect model was defined as the period between the 1st June and harvest. The date 1st of June was chosen in the aim to cover each accession growing period because the first harvest date is late June. On this date, all the accessions studied are on fruit setting and begin to grow regardless the harvest date.

The climate features were the total precipitation (mm), mean, maximum and minimum daily temperatures (ºC), mean daily temperature range (ºC), mean daily relative humidity (%) and mean daily solar radiation (W m^{-2}). Both the dependent and independent variables were centered and scaled to a common range so the model coeffients could be compared. A stepwise procedure based on the Akaike Information Criterion (AIC) statistic was used to select between alternative model configurations with different independent variables. The model coefficients were fitted by the maximum likelihood method, as implemented in the nlme package for R (Pinheiro et al., 2017).

A cluster analysis was applied to the accessions on the model coefficients' space, in order to determine groups of accessions with similar relationship between climate and biochemical traits. The cluster analysis was based on the Ward's D method based on the Euclidean distance (Ward et al., 1963).

2.7. Chemicals

All chemicals were of analytical grade. Aluminium chloride ($AlCl_3$), 3,4,5 trihydroxybenzoic acid (gallic acid), sodium carbonate (Na_2CO_3) and sodium hydroxide (NaOH) were purchased from PanReac Quimica SA (Barcelona, Spain). The bipyridyl, cathequin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), folin-ciocalteau's reagent, phosphoric acid (H_3PO_4), iron (III) chloride ($FeCl_3$), metaphosphoric acid (HPO_3), sodium nitrite ($NaNO_2$) and 6-hydroxy-2,5,7,8-

tetramethylchromane-2-carboxylic acid (trolox) were purchased from Sigma-Aldrich (Saint Louis, MO, USA).

3. Results

3.1. Accession and year effect

The statistical analysis was carried out with the 155 accessions presented in the Table 1. The ANOVA analysis showed significant differences ($P \leq 0.001$) among the different apple accessions for each year and for all traits evaluated (Figure 1). Additionally, statistical significance differences were also obtained for the interaction between accession and year for all traits studied ($P \leq 0.001$) (Supplementary Files 1 and 2).

Differences on average daily temperature (tmd), average minimum daily temperature (tmn), average maximum daily temperature (tmx) and mean daily temperature range (trg), as well as solar radiation (rad), mean daily relative humidity (rh) and total precipitation (pre_tot) during the five years of the study were also found (Supplementary File 1). The 2018 climatic traits during the fruit growth period for each accession (from 1st June to harvest date) varied less than the other years of the study. Noneless, 2016 was the year with less precipitation and lower levels of relative humidity for the major part of the accessions. Meanwhile, 2017 seemed to be the year with more precipitation and higher relative humidity. Nevertheless, the values for rh in 2018 were even higher compared to the other four years (2014 – 2017). The years 2015 and 2017 were the hottest with higher values for tmd, tmn and tmx, while the 2014 was the coldest. The temperatures (tmd, tmx, tmn and trg) for 2014 were very similar for all the accession growing periods studied. According to the solar radiation, 2014, 2015 and 2017 had similar profiles for the growing periods of the different accessions, while solar radiation values tended to be lower in 2018.

3.2. Basic fruit quality traits, biochemical compounds and antioxidant activity

The soluble solids content (SSC) ranged among apple accessions and years from 8.5 ('De Agosto – MRF 57', in 2017) to 20.3 ('Pera_2', in 2017) ºBrix. Regarding the titrable acidity (TA), values varied greatly ranging from 1.1 ('Verde Doncella – MRF 36', in 2014) to 20.1 ('Urarte', in 2015) g malic acid per liter. Ripening index (RI=SSC/TA) values ranged from 0.5 ('Urarte', in 2015) to 12.3 ('Verde Doncella – MRF 36', in 2014). The standard deviation for SSC, TA and RI respectively were fitted at 1.9, 3.3 and 1.5 (Table 2). According to the mean of the five years of study (Supplementary file 3), SSC ranged from 10.1 ('Bellaguarda Lardero – MSV 27') to 17.8 ('Terrera') ºBrix, TA varied from 1.8 ('Verde Doncella – MRF 36') to 17.3 ('Reguard_2 – MRF 54') g malic acid per liter and RI varied from 0.7 ('Urarte') to 8.6 ('Verde Doncella – MRF 36').

The total phenolics content (TPC) varied greatly among apple accessions and years ranging from 3.3 ('Poma de San Juan', in 2018) to 116.7 ('Camuesa Fina de Aragón', in 2015) mg gallic acid equivalents (GAE)/100 g FW. For the total flavonoids content (TFC), values ranged from

0.7 (‘Poma de San Juan’, in 2018) to 142.1 (‘Camuesa Fina de Aragón’, in 2014) mg catechin equivalents (CE)/100 g FW. Regarding AsA (ascorbic acid – Vitamin C), values ranged from 0.4 (‘Delgared infel’, in 2016) to 13.2 (‘Transparente’, in 2014) mg AsA/100 g FW. Finally, relative antioxidant capacity (RAC) values ranged from 1.7 (‘Poma de San Juan’, in 2018) to 44.6 (‘Les_1 – MRF 73’, in 2014) mg trolox/100 g FW. The standard deviation for TPC, TFC, AsA and RAC were fitted at 19.4, 18.4, 1.6 and 6.6 respectively (Table 2). Regarding the mean of the five years of study (Supplementary File 3), TPC varied from 15.2 (‘Biscarri_1 – M 107’) to 98.1 (‘Camuesa Fina de Aragón’) mg gallic acid equivalents (GAE)/100 g FW, TFC ranged from 6.0 (‘Biscarri_1 – M 107’) to 89.0 (‘Camuesa Fina de Aragón’) mg catechin equivalents (CE)/100 g FW. According to AsA, values ranged from 1.4 (‘Delgared infel’) to 5.9 (‘Reguard_1 – MRF 53’) mg AsA/100 g FW. Finally, RAC varied from 5.9 (‘Delgared infel’) to 30.8 (‘Les_1 – MRF 73’).

Comparison of means for the different basic quality traits, biochemical compounds and antioxidant capacity (Figure 2), showed no significant differences between foreign and local accessions for SSC and RI. Nevertheless, several foreign accessions such as ‘Granny Smith_1’, ‘Granny Smith_2’, ‘Reineta Gris’ or ‘Redaphough’ showed lower levels of acidity (TA) than the local ones as the ‘Reguard_2 – MRF 54’, ‘Urarte’, ‘Bossost_2 – MRF 76’ and the ‘Transparente Blanca’ accessions. Regarding the bioactive compounds and the RAC, all traits showed significant differences between foreign and local accessions. Indeed, for all the parameters, foreign cultivars such as ‘Akane’, ‘Deljeni’, ‘Delorgue Festival’, ‘Reineta Gris’, among others, had lower values than local accessions such as ‘Camuesa Fina de Aragón’, ‘Les_1 – MRF 73’, ‘Peruco de Caparroso’, and ‘Prau Riu_5’, among others.

3.3. Pearson’s correlations

Significant ($P \leq 0.01$) bilateral correlations were found between all traits evaluated (Table 3). As expected, RAC was highly and positively correlated with TPC (r=0.901) and TFC (r=0.865). TPC was also significantly and highly positively correlated with TFC (r=0.963). In a minor proportion, AsA showed a significant and moderate positive correlation with TPC (r=0.415). Also, significant moderate positive correlations were found between TA and TFC (r=0.464) and TPC (r=0.450).

Table 4 shows the Pearson’s correlation between the different climatic traits studied. Thus, tmd was highly and positively correlated with tmx (r=0.979), tmn (r=0.948) and trg (r=0.645). The mean daily temperature range (trg) was also highly correlated with tmx (r=0.780). Solar radiation (rad) was positively correlated with tmx (r=0.678), tmn (r=0.582), tmd (r=0.699) and trg (r=0.541). Relative humidity (rh) showed negative correlation with tmx (r=-0.739), tmd (r=-0.664) and trg (r=-0.822). Solar radiation was also highly correlated but in a negative way with rh (r=-0.777). Finally, the total precipitation showed a positive correlation with the rh (r=0.591) but negative with the solar radiation (r=-0.772). These correlations helped to the choice of the more significant parameters useful for the mixed-effects model and for the two-way hierarchical agglomerative cluster analysis.

3.4. Principal components analysis

A principal component analysis (PCA) was carried out to understand how traits could segregate the different accessions studied (Figure 3). The first two PCs, PC1 and PC2, accounted respectively for 50.5% and 21.3% of the total variability (Supplementary File 4). Indeed, a total of 71.8% of the variance could be explained accounting with only the two first PCs. The PC1 mainly contributed to biochemichal traits (TPC, TFC, RAC and AsA) (Supplementary File 5). Accessions on the positive side of PC1 corresponding mainly to local accessions, induced, in general, higher values of those biochemical compounds (for instance 'Camuesa Fina de Aragón', 'Transparente', and 'Les-1'). In contrast, accessions on the negative side of PC1, corresponding to most of the foreign cultivars (for instance 'Nueva Starking', 'Averdal' and 'Evasni'), showed, in general, lower values for those biochemical compounds. Indeed, only eight foreign accessions were situated on the positive side of the PC1 ('Akane', 'Astrakan Red', 'Cox's Orange Pippin', 'Deljeni', 'Granny Smith_1', 'Granny Smith_2', 'Reineta Blanca del Canadá_2' and 'Reineta Gris').

Moreover, the PC2 loadings suggested that separation on this component was mainly due to basic fruit quality parameters (TA, SSC and RI) (Supplementary File 5). On the negative side of PC2, accessions showed higher values for TA (for instance 'Reguard-2' and 'Urarte'). A pool of foreign accessions with specific fruit quality values could be also identified through PCA analysis. The 'Granny Smith' cultivar seem to be the more acidic foreign accession according to the PCA, as it is commonly known. Nevertheless, the more acidic accession was 'Reguard-2 - MRF 54' which is a Spanish local accession. The 'Granny Smith_1' is the first most acidic foreign accession but there are 22 local accessions with higher TA values (Supplementary File 3).

3.5. Mixed-effect model and relationships between accessions, climate and genetic origin

Based on the highest correlations between the climatic traits above mentioned and a stepwise procedure based on the Akaike Information Criterion (AIC) statistic, the best significant model (lowest AIC) for the TPC analysis included the following three variables: tmn, trg and rad. The random effects indicated that there were differences between accessions in both their intercepts (mean TPC values) and climate coefficients. For the TFC analysis, the best significant model (lowest AIC) included pre, rad and tmn. The best significant models for RAC, SSC and TA analysis included rad and tmn. Regarding the AsA analysis, the best significant model included pre, rad and trg. Finally, the RI analysis model included pre and rad. The results of the mixed-effects models were significant for all variables (basic fruit quality traits, antioxidant compounds and relative antioxidant capacity), demonstrating that there is a close relationship between the evaluated fruit traits and the climate characteristics of the growing season.

A two-way hierarchical agglomerative cluster analysis was used to classify the 155 apple accessions studied into groups according to their model coefficients (Figure 4). Clusters of accessions would therefore indicate a similar relationship between antioxidant traits and climate, while clusters of variables would group model coefficients that tended to behave similarly across the accessions.

Concerning the effect of climate traits, there were small differences between clusters regarding the effect of solar radiation, with an overall positive effect for TPC and AsA in all cases (shown in red colors in Figure 4) and negative effect for RI. Similarly, temperature tended to have a negative effect on TPC, TFC, and AsA, while the results were more mixed for other variables. Precipitation had an overall negative effect on RI, although lower than the other climate variables. The main effect of high temperature was to reduce the phenolics and flavonoids content and RAC, while solar radiation had a positive and strong influence on all of them. Table 5 shows the mean for each cluster; meanwhile the Table 6 considers whether the accession is a local or a foreign one. It is easily noticed that the foreign accessions showed in general lower values for TPC, TFC, RAC, AsA, SSC, TA and RI. As previously said for the TPC and AsA, solar radiation had a great and positive effect indepently of the cluster. However, solar radiation showed different profiles whether the accession was local or foreign for TFC, RAC and SSC (Table 6).

Two big clusters were identified in the accessions axis, and a separation into five smaller clusters was selected as optimum (Figure 4). The clusters were numbered according to the height at which they separate from the remaining group. The first three clusters included respectively 19, 33 and 17 accessions; the fourth cluster grouped 34, while the last one included 52 accessions. There seemed to exist a relationship between the clusters and the origin of the accession. Thus, clusters 1, 2 and 3 included mostly local accessions (64 out of 69 accessions). Clusters 4 and 5, included 51 out of the 56 foreign accessions present in the collection. With regard to the ploidy of the accessions studied, more than 75% of the triploid accessions were grouped in the first major cluster which included clusters 1 and 2.

Regarding the independent variables, the hierarchical classification tended to group together the intercepts of the models, related to the mean values of the biochemical traits, and then the climatic variables. Accessions from clusters 1 and 2 had similar profiles for the intercept of basic fruit quality traits (TA and RI) and antioxidant parameters (TPC, TFC, RAC, AsA), which were both very high. Cluster 3 had very high antioxidant profiles as clusters 1 and 2, but instead it had low values of the basic fruit quality traits as the fourth cluster. Cluster 4 had low coefficients for both fruit quality traits and antioxidants, while cluster 5 was characterized by relatively low correlations profiles for antioxidants and more mixed profiles for basic quality traits. The profiles for ascorbic acid were more mixed, athough some clusters had consistently low (Cluster 4) or high (clusters 1, 2 and 3) profiles. A similar situation was found for the SSC.

4. Discussion

In the present study, basic fruit quality traits (TA, SSC and RI) and biochemical compounds (TPC, TFC, AsA), as well as antioxidant capacity (RAC), were evaluated on 155 apple cultivars for five years (2014-2018). As expected, the levels of the assessed traits differed greatly among accessions and years, as demonstrated in previous studies (Boyer and Liu, 2004; Reig et al., 2015; Van Der Sluis et al., 2001), indicating that both accession and year had consistent effects on the evaluated parameters. Significantly, other studies in peach (Font i Forcada et al., 2014b, 2019a, Iglesias et al., 2019) and apricot (Gómez-Martínez et al., 2021) also reported a year effect due to the climatic parameters that affected fruit quality, biochemical compounds and relative antioxidant capacity. Values were, in general, within the range reported for apple cutivars for the basic fruit quality traits (Guan et al., 2015; Reig et al., 2015; Slatnar et al., 2019; Wu et al., 2007; Zhen et al., 2018), as well as for the antioxidant biochemical compounds (Boyer and Liu, 2004; Slatnar et al., 2019; Yuri et al., 2009). However, in the present work, the range varied in a higher extent due to the extremely different plant material studied, probably because of different genetic background in comparison to commercial cultivars. In fact, this is the first study reporting fruit quality, bioactive compounds levels and relative antioxidant capacity profile of these accessions for a long period of time (five years) and relating these parameters to the climatic conditions. It is interesting to note that the local accessions ‘Camuesa Fina de Aragón’, ‘Les_1 – MRF 73’, ‘Prau Riu_5’ and ‘Transparente’, showed higher values than expected for TPC, TFC and RAC. In fact, the first twenty accessions ranking with higher TPC, TFC and RAC values were local. With regards to the foreign accessions, ‘Akane’, ‘Deljeni’, ‘Delorgue Festival’, ‘Granny Smith_1’, ‘Granny Smith_2’, ‘Red Rome Beauty’, ‘Reineta Blanca Canada_2’ and ‘Reineta Gris’ had the highest content of TPC, TFC and RAC, but, in general, they had 1.5-2 times lower values than those presented by ‘Camuesa Fina de Aragón’, ‘Les_1 – MRF 73’, ‘Prau Riu_5’ and ‘Transparente’.

The PCA analysis of the 155 accessions demonstrated a great variability between accessions. It also showed that foreign accessions were more closely related between them, constituting a foreign genetic pool. The PCA results showed that foreign accessions can be segregated in a single group because they shared similar values for the traits studied. However, local accessions can not be segregated in a single group, as they have some really different profiles, being diffuse in the hole graph. Pereira-Lorenzo et al. (2017) studied a large set of apple genotypes conserved in different Spanish collections using microsatellite markers. They discriminated an Iberian genepool of apple accesions from a wide set of foreign cultivars. Similarly, in the present work, we discriminated, in the PCA results, two groups in accordance with the genetic diversity of the Spanish core collection proposed by Pereira-Lorenzo et al. (2017). In the two-way hierarchical analysis based on the Euclidean distance we could find these two different groups whether the accession is local or foreign too. Indeed, clusters 1, 2 and 3 included 64 local accessions out of 69 total accessions and clusters 4 and 5, grouped 51 out of the 56 foreign accessions present in the collection.

The health benefits could be one of the main attributes to promote fruit consumption. Many studies have shown that antioxidant compounds are among the most benefic biomolecules ingested in fruit consumption (Boeing et al., 2012; Boyer et al., 2004; Lattanzio, 2013). This study showed lower values for TA for most of the foreign accessions and higher values for SSC and thus for RI. This fact leads us to hypothesize that foreign accessions or commercial cultivars had the acidity (TA) balanced by the sweetness (SSC) in the aim of the acceptance by customers, as it has also been proposed by other studies (Jakobek et al., 2020; Musacchi et al., 2018). Nevertheless, as the acidity was correlated moderately but significantly with the biochemical compounds, this could indicate that taste and the organoleptic properties of pulp were ranked first on the past breeding programs beyond health benefits provided by antioxidants (Gómez-Martínez et al., 2021; Preti & Tarola, 2020).

Interestingly, the two-way cluster analysis showed apparently no relationship between the different clusters and harvest date or skin color. All studied genotypes had similar tree management and were grown under the same climatic and soil conditions. Actually, the climatic conditions for a given year only differed between cultivars due to the different lengths of their growing periods, controlled by the harvest date. It must be noted that the variability range of the climatic conditions was limited to between-years variation, as the experiment was carried out in one single location. There is a much wider range of variability to be expected if the experiment was carried out in a variety of location of contrasting environmental conditions (climate), which could possibly lead to a stronger climatic variability than found here.

The year effect should be explained by the climatic variables (temperatures, precipitation, radiation, etc.), although it is difficult to explain the individual contributions of the various possible environmental factors (Lattanzio, 2013). Actually, in addition to a direct impact on metabolite levels (basic fruit quality traits and antioxidant biosynthesis), environmental conditions will also affect general fruit physiology and development (Davey et al., 2007). Light and temperature affect many fruit quality traits, including sugar nutrient levels, texture, taste and flavour (Davey et al., 2007; Fischer et al., 2016). Apples contain several antioxidants originated from different pathways and accumulated in the fruit as a consequence of sun exposure (Lattanzio, 2013). It is well known that flavonoids are an important group of phenolic compounds in apples and both contribute significantly to the relative antioxidant capacity of fruits (Bui et al., 2019; Lattanzio, 2013; Marks et al., 2007). Previous studies in apples reported similar positive correlations between TPC, TFC, and RAC, as found in this work (Cocci et al., 2006; Preti & Tarola, 2020; Raudone et al., 2017; Vieira et al., 2009; Wang et al., 2015). The total phenolics are the main compounds responsible for keeping fruits from UV/high light damage (Li et al., 2013). This accumulation of phenolics might be related to the photoprotective function of phenolics under high light and UV irradiation (Lattanzio, 2013; Li et al. 2008). Agroclimatic traits also influenced bioactive compounds values (AsA, TFC, TPC and RAC), in general, by increasing their levels, as previously demonstrated in other studies with apple cultivars (Yuri et al., 2009; McGhie et al., 2005). Furthermore, Li et al. (2013) showed that Gallic acid content became higher in the sun-exposed peel as fruit developed. Our work shows a positive effect of solar radiation in the flesh fruit by increasing the biochemical compound levels for AsA, TFC and TPC. In the case of the RAC, solar radiation increased their values for the local accessions, but decreased them for the foreign cultivars. In the present study, solar radiation exhibited also a high contribution to the AsA values in flesh tissue for all the studied accessions. In contrast, previous works demonstrated that solar radiation only influenced the AsA values in the apple peel and not in the apple flesh (Li et al., 2009). In some works, a higher accumulation of AsA was found in the sun-exposed side compared to the shaded side (Bui et al., 2019). However, the exact impact of solar radiation in AsA contents in apple flesh fruit is not clear yet, as the available studies and cultivars evaluated show contradictory results with the present work (Bui et al., 2019; Li et al., 2009). Nevertheless, few cultivars have been studied, and very few multi-year studies exist. The same occurs with the temperature traits. Davey et al. (2007) showed a negative correlation between average preharvest daytime temperature and AsA values in whole fruits, whereas Łata (2007), found a significantly higher total amount of AsA in the warmer year of their study. In the present work, considering five years and 155 accessions, a significant effect between lower temperatures and higher biochemical compound values was obtained. Lattanzio et al. (2001) demonstrated that the low temperature effect involved a cold-induced stimulation of the phenylalanine ammonia-lyase (PAL) activity in apple as well as other enzymes important in the phenolic biosynthetic pathway.

Biochemical compounds play an important role in postharvest prevention of several diseases (Chagné et al., 2019; Davey et al., 2007). Indeed, different links exist between crop antioxidant defence, maintenance of postharvest fruit quality and harvest date (Davey et al., 2007). Piretti et al. (1994) found that the concentration of antioxidant compounds decreased during storage in ‘Granny Smith’ apple cultivar. Bui et al. (2019) showed that higher exposure of apple fruit to sun in the field might lead to an improved tolerance to postharvest fungi infection by increasing the antioxidant superoxide dismutase (SOD) and ascorbate peroxidase (APX) enzyme activity. The general accumulation and increase in TPC, TFC, AsA and higher RAC values due to solar radiation, demonstrated in the present work, highlight the positive effect of radiation on the bioactive compounds. Besides this positive effect, local accessions seem to be more affected than the foreign ones, demonstrating the importance of the local fitogenetic resources and their adaptation to the conditions prevailing in the central Ebro Valley (Reig et al., 2015). In fact, some of the antioxidant compounds might have antifungal activity too, as the flavonoid, quercetin-3-galactoside reported by Bui et al. (2019) Both higher content of phenolic compounds (Li et al., 2013) and higher activities of key enzymes involved in the antioxidant metabolism (Davey et al., 2007, Lattanzio et al., 2001) suggest that the polyphenolic metabolism in apples is upregulated by high irradiance (Lattanzio, 2013; Li et al., 2013).

Finally, statistical analyses highlighted apple accessions, year and climate traits as main factors affecting metabolite profiles and fruit quality characteristics. Our work emphasizes that research could lead to the development and selection of cultivars (Pereira-Lorenzo et al., 2017; Verma et al., 2019) having the additional benefit of improved nutritional value (Font i Forcada et al., 2019a; Yuri et al., 2009) for the consumer. Indeed, accessions that have a higher content of antioxidants can be selected to promote their positive effect on health (Boeing et al., 2012; Boyer et al., 2004), and can be selected to be consumed in different ways (fresh, juice, dry snacks, etc.). Moreover, application of high-throughput phenotyping techniques in the germplasm collection, in combination with SSRs (Pereir-Lorenzo et al., 2017) or high-density SNP genotyping (Font i Forcada et al., 2019b; Vanderzande et al., 2019), could offer additional valuable information in interpreting the genetic control of fruit quality traits in apple.

5. Conclusions

This study shows a considerably high biodiversity in the apple germplasm studied for the content of bioactive compounds and basic fruit quality traits. We found a higher average content of antioxidants in the local accessions as compared with the foreign ones, which should increase awareness of the importance of the local phytogenetic resources. Moreover, we found that climatic traits such as precipitation, solar radiation, and temperature strongly influenced the antioxidant and metabolite profiles of the different accessions studied. The bioactive compound values tended to decrease, in general, with higher temperatures, while they increased with precipitation and solar radiation. Albeit the limited range of variation of climatic conditions in our study (as only between-year variation in a single location was considered), this significance of the climatic parameters highlights the importance of the geographic region where the crop is cultivated in the resulting bioactive compounds. Genetic progress could be greatly affected by selecting the right accessions on future breeding programs to improve biochemical characteristics desired of fruits depending on the area of the crop.

Acknowledgements

We would like to thank María Pilar Soteras, Rosa Giménez and Fernando Gómez for technical assistance in fruit quality analysis and helping in plant management in the orchard. The authors are grateful to Dr. Javier Abadía, María Pilar Vallés and Ana María Castillo at EEAD-CSIC, Spain, for providing equipment access.

Fundings

This study was funded by the Spanish Ministry of Science Innovation and Universities grants RFP 2012-00020, RFP 2015-00019 and PIE201640E070, cofounded by FEDER, and the Regional Gov. of Aragon (A44, T07_17R, and P. Mignard grant).

Competing interests: The authors declare no competing financial interest.

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Tables and figures

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Table 5. Mean values of the traits studied for each cluster for the 155 accessions.

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Figure 4. Two-way hierarchical analysis of the 155 accessions based on the Euclidean distance on the model coefficients space. Apple accessions are grouped into five groups according to the dendrogram. Positive and negative coefficients indicate the intercept (int) and the effect of climate variables (minimum temperature, tmn; temperature range, trg; precipitation, pre; radiation, rad) on different biochemical traits (TA, titrable acidity; RI, ripening index; TPC, total phenolic content; RAC, relative antioxidant content; AsA, ascorbic acid). Additionally, values of auxiliary variables not used in the cluster analysis are also shown: skin color, harvest date (in julian days), and origin.

Tables and figures

Table 1. Information of the 155 apple accessions used on this study.

Accession	Nº	Code EEAD	Classification	Origin	Skin color	Ploidy
Aciprés	1	3339 AD	Local	Huesca, SP	Bicolor	2
Akane	2	2902 AD	Foreign	Japan	Bicolor	2
Almenar_2 - MRF 46	3	3555 AD	Local	Lérida, SP	Bicolor	2
Ascara_1	4	3423 AD	Local	Huesca, SP	Bicolor	2
Ascara_2	5	3424 AD	Local	Huesca, SP	Bicolor	2
Astrakan Red	6	3378 AD	Foreign	Rusia	Bicolor	2
Audiena de Oroz	7	3375 AD	Local	Navarra, SP	Green	2
Augüenta	8	3335 AD	Local	Lugo, SP	Green	2
Averdal_1	9	882021	Foreign	-	Red	2
Averdal_2	10	892340	Foreign	-	Red	2
Baujade	11	923284 AD	Foreign	France	Green	2
Bellaguarda Lardero - MSV 27	12	3547 AD	Local	La Rioja, SP	Yellow	2
Belleza de Roma	13	638 AD	Foreign	Italy	Bicolor	2
Biscarri_1 - M 107	14	3726 AD	Local	Lérida, SP	Bicolor	2
Blackjon	15	2690 AD	Foreign	Wenatchee, USA	Bicolor	2
Bofla	16	3418 AD	Local	La Rioja, SP	Green	2
Boluaga	17	3340 AD	Local	Guipúzcoa, SP	Bicolor	3
Bossost_1 - MRF 75	18	3626 AD	Local	Lérida, SP	Bicolor	3
Bossost_2 - MRF 76	19	3627 AD	Local	Lérida, SP	Brown	3
Bossost_4 - MRF 78	20	3629 AD	Local	Lérida, SP	Bicolor	2
Bossost_5 - MRF 79	21	3630 AD	Local	Lérida, SP	Bicolor	3
Bost Kantoia	22	3341 AD	Local	Guipúzcoa, SP	Yellow	2
Cabdellà_2 - MRF 49	23	3613 AD	Local	Lérida, SP	Bicolor	2
Cabello de Angel	24	3255 AD	Local	Calatayud, SP	Yellow	2
Calvilla de San Salvador	25	3342 AD	Local	Zaragoza, SP	Bicolor	2
Camosa - MRF 42	26	3553 AD	Local	Lérida, SP	Bicolor	2
Camosa - MRF 60	27	3620 AD	Local	Lérida, SP	Bicolor	2
Camuesa de Daroca	28	3371 AD	Local	Zaragoza, SP	Green	2
Camuesa de Llobregat	29	1342 AD	Local	Barcelona, SP	Green	2
Camuesa Fina de Aragón	30	3372 AD	Local	Huesca, SP	Bicolor	2
Carapanón	31	3634 AD	Local	Asturias, SP	Bicolor	3
Carrió	32	3636 AD	Local	Asturias, SP	Bicolor	3
Cella	33	2512 AD	Local	Teruel, SP	Green	2
Ciri Blanc	34	3402 AD	Local	Gerona, SP	Green	2
Cirio - MRF 52	35	3615 AD	Local	Gerona, SP	Green	2
Cox's Orange Pippin	36	2889 AD	Foreign	England	Bicolor	2
Cripps Pink	37	933540 AD	Foreign	England	Bicolor	2
Cuallarga	38	3467 AD	Local	Gerona, SP	Green	2
Cul de Cirio - MRF 39	39	3551 AD	Local	Lérida, SP	Bicolor	2
De Agosto - MRF 57	40	3619 AD	Local	Lérida, SP	Bicolor	2
De Pera	41	3416 AD	Local	La Rioja, SP	Yellow	2
De Valdés	42	3632 AD	Local	Asturias, SP	Bicolor	2
Delciri	43	3413AD	Local	Baleares, SP	Yellow	2
Delcon	44	2896 AD	Foreign	USA	Bicolor	2
Delgared Infel	45	902708 AD	Foreign	-	Red	2
Deljeni	46	851305 AD	Foreign	Malicorne, France	Yellow	2
Delkistar	47	923273 AD	Foreign	USA	Bicolor	2
Delorgue Festival	48	913044 AD	Foreign	Malicorne, France	Bicolor	2
Elista	49	912883 AD	Foreign	Netherlands	Bicolor	2
Esperiega	50	3420 AD	Local	La Rioja, SP	Yellow	2
Esperiega de Olba - M 106	51	3725 AD	Local	Teruel, SP	Bicolor	2
Eugenia	52	3468 AD	Local	Gerona, SP	Bicolor	2
Evasni - Scarlet Spur	53	933554	Foreign	France	Bicolor	2

Florina	54	3633 AD	Foreign	Angers, France	Bicolor	2
Fuji	55	3488 AD	Foreign	Japan	Bicolor	2
Gala	56	3197 AD	Foreign	New Zeland	Bicolor	2
Galaxy	57	892451 AD	Foreign	New Zeland	Bicolor	2
Golden Delicious_675	58	675 AD	Foreign	-	Yellow	2
Golden Delicious Infel_972	59	2491 AD	Foreign	France	Yellow	2
Golden Paradise	60	3739 AD	Foreign	Spain	Yellow	2
Golden Smoothee	61	3286 AD	Foreign	West Virginia, USA	Yellow	2
Granny Smith_1	62	2614 AD	Foreign	Australia	Green	2
Granny Smith_2	63	3196 AD	Foreign	Australia	Green	2
Guillemes	64	3411 AD	Local	Baleares, SP	Bicolor	2
Hared	65	892232 AD	Foreign	France	Bicolor	2
Helada	66	3368 AD	Local	Baleares, SP	Green	2
Hierro	67	3374 AD	Local	Navarra, SP	Bicolor	2
Idared	68	2484 AD	Foreign	Idaho, USA	Bicolor	2
Irgo_2 - MRF 66	69	3622 AD	Local	Lérida, SP	Bicolor	2
Jonadel	70	2650 AD	Foreign	Iowa, USA	Bicolor	2
Jonagored	71	882001 AD	Foreign	Halen, Belgium	Bicolor	3
Jonathan_1	72	2495 AD	Foreign	New York, USA	Bicolor	2
Jonathan_2	73	3096 AD	Foreign	New York, USA	Bicolor	2
Jubilee	74	851304 AD	Foreign	Middlesex, England	Bicolor	2
Landetxo	75	3343 AD	Local	Navarra, SP	Bicolor	2
Les_1 - MRF 73	76	3624 AD	Local	Lérida, SP	Bicolor	2
Les_2 - MRF 74	77	3625 AD	Local	Lérida, SP	Bicolor	2
Mañaga	78	469 AD	Local	Huesca, SP	Green	2
Mañaga - MRF 43	79	3554 AD	Local	Lérida, SP	Bicolor	2
Marinera	80	3412 AD	Local	Baleares, SP	Bicolor	2
Marquinez	81	3419 AD	Local	La Rioja, SP	Bicolor	3
McIntosh	82	3192 AD	Foreign	Canada	Bicolor	2
Médulas_1 - MSV 38	83	3548 AD	Local	Lérida, SP	Bicolor	2
Melrose	84	2482 AD	Foreign	Ohio, USA	Bicolor	2
Merrigold	85	851307 AD	Foreign	France	Yellow	2
Montcada_1 - MRF 82	86	3631 AD	Local	Lérida, SP	Bicolor	2
Morro de Liebre	87	3256 AD	Local	Zaragoza, SP	Bicolor	2
Nesple	88	3410 AD	Local	Baleares, SP	Bicolor	2
Normanda	89	3252 AD	Local	Zaragoza, SP	Bicolor	3
Nueva Starking	90	1899 AD	Foreign	-	Red	2
Ortell	91	413 AD	Local	Zaragoza, SP	Bicolor	3
Ortell - MSV 24	92	3546 AD	Local	La Rioja, SP	Bicolor	2
Pera_2	93	3417 AD	Local	La Rioja, SP	Yellow	2
Pera de Sangüesa	94	3379 AD	Local	Navarra, SP	Green	3
Peromingan	95	1158 AD	Local	Asturias, SP	Green	2
Pero Pardo	96	3369 AD	Local	Navarra, SP	Green	3
Peruco de Caparroso	97	3373 AD	Local	Navarra, SP	Bicolor	2
Plaona	98	923283 AD	Foreign	-	Green	2
Poma de San Juan - MRF 47	99	3556 AD	Local	Lérida, SP	Bicolor	2
Prau Riu_3	100	3491 AD	Local	Asturias, SP	Bicolor	2
Prau Riu_4	101	3492 AD	Local	Asturias, SP	Bicolor	3
Prau Riu_5	102	3493 AD	Local	Asturias, SP	Green	2
Prima	103	851306 AD	Foreign	Illinois, USA	Red	2
Rebellón	104	3370 AD	Local	Navarra, SP	Bicolor	2
Red Delicious	105	3085 AD	Foreign	USA	Bicolor	2
Red Elstar	106	882002	Foreign	Netherlands	Bicolor	2
Red Rome Beauty	107	2897 AD	Foreign	Ohio, USA	Bicolor	2
Redaphough	108	933411 AD	Foreign	USA	Red	2
Red Chief	109	851308 AD	Foreign	USA	Bicolor	2
Regal Prince_1	110	882022 AD	Foreign	France	Bicolor	2
Regal Prince_2	111	892341 AD	Foreign	France	Bicolor	2
Reguard_1 - MRF 53	112	3616 AD	Local	Lérida, SP	Bicolor	2
Reguard_2 - MRF 54	113	3617 AD	Local	Lérida, SP	Bicolor	2

Reguard_4 - MRF 56	114	3618 AD	Local	Lérida, SP	Bicolor	2
Reina de Reinetas	115	2488 AD	Foreign	Netherlands	Bicolor	3
Reineta Blanca Canada_1	116	308 AD	Local	Zaragoza, SP	Green	3
Reineta Blanca Canada_2	117	3111 AD	Foreign	France	Green	3
Reineta Blanca Canada_3	118	3194 AD	Local	Zaragoza, SP	Green	3
Reineta Encarnada	119	3635 AD	Local	Asturias, SP	Bicolor	2
Reineta Gris	120	2883 AD	Local	Spain	Brown	3
Reineta Inesita Asua	121	2543 AD	Local	Bilbao, SP	Bicolor	3
Reineta Regil	122	3466 AD	Local	Vizcaya, SP	Green	3
Reneta	123	3408 AD	Local	Mallorca, SP	Bicolor	2
Roja Valle Benejama	124	1038 AD	Local	Valencia, SP	Bicolor	2
Roser de la Reula - MRF 40	125	3552 AD	Local	Lérida, SP	Bicolor	2
Royal Red Delicious	126	2363 AD	Foreign	USA	Bicolor	2
Rubinete	127	861526 AD	Foreign	Switzerland	Bicolor	2
Ruixou_1 - MRF 51	128	3614 AD	Local	Lérida, SP	Bicolor	2
San Felipe	129	3376 AD	Local	Navarra, SP	Bicolor	2
San Miguel	130	2579 AD	Local	La Rioja, SP	Bicolor	2
Sandia	131	3336 AD	Local	Lugo, SP	Bicolor	2
Sant Jaume	132	3470 AD	Local	Gerona, SP	Bicolor	3
Sant Joan	133	3409 AD	Local	Mallorca, SP	Bicolor	2
Santa Margarida	134	3401 AD	Local	Gerona, SP	Bicolor	3
Signatillis	135	3403 AD	Local	Gerona, SP	Green	2
Solafuente	136	3559 AD	Local	Cantabria, SP	Bicolor	3
Starking_1	137	2964 AD	Foreign	USA	Bicolor	2
Starking_2	138	632 AD	Foreign	USA	Bicolor	2
Starkrimson_1	139	1904 AD	Foreign	USA	Red	2
Starkrimson_2	140	3195 AD	Foreign	USA	Red	2
Taüll_1 - MRF 67	141	3623 AD	Local	Lérida, SP	Green	2
Telamon	142	3398 AD	Foreign	Kent, England	Bicolor	2
Tempera	143	3334 AD	Local	Lugo, SP	Green	2
Terrera	144	3469 AD	Local	Gerona, SP	Brown	3
Top Red Delicious	145	2651 AD	Foreign	USA	Red	2
Totxa	146	3471 AD	Local	Gerona, SP	Green	2
Transparente	147	3377 AD	Local	Navarra, SP	Green	2
Transparente Blanca	148	3344 AD	Local	Navarra, SP	Yellow	2
Urarte	149	3415 AD	Local	La Rioja, SP	Green	3
Urtebete	150	3345 AD	Local	Navarra, SP	Green	2
Valsaina	151	3558 AD	Local	Cantabria, SP	Bicolor	2
Verde Doncella - MRF 36	152	3549 AD	Local	Teruel, SP	Green	2
Verde Doncella_1	153	2125 AD	Local	Zaragoza, SP	Green	2
Verde Doncella_2	154	310 AD	Local	Zaragoza, SP	Green	2
Vinçada Tardía - MRF 61	155	3621 AD	Local	Lérida, SP	Green	3

AD, Aula Dei; EEAD, Experimetal Station of Aula Dei; SP, Spain; USA, United States of America

Table 2. Basic statistics of fruit quality traits, biochemical compounds and antioxidant capacity over the accessions and years of the study: units, number of observed accessions (n), minimum, maximum, mean values, and standard deviation (SD).

Trait	Units	n	Minimum	Maximum	Mean	SD
SSC	ºBrix	155	8.5	20.3	13.5	1.9
TA	g malic acid L-1	155	1.1	20.1	6.8	3.3
RI	-	155	0.5	12.3	2.9	1.5
TPC	mg GAE 100 g FW-1	155	3.3	116.7	39.7	19.4
TFC	mg CE 100 g FW-1	155	0.7	142.1	23.1	18.4
AsA	mg AsA 100 g FW-1	155	0.4	13.2	2.8	1.6
RAC	mg Trolox 100 g FW-1	155	1.7	44.6	15.4	6.6

SSC, soluble solids content; TA, titratable acidity; RI, ripening index; TPC, total phenolics content; TFC, total flavonoids content; AsA, Ascorbic acid; RAC, relative antioxidant content.

Table 3. Pearson's correlation coefficients between traits.

	TFC	TPC	AsA	TA	RI
RAC	0.865**	0.901**	0.369**	0.282**	ns
TFC		0.963**	0.386**	0.450**	-0.315**
TPC			0.415**	0.464**	-0.296**
AsA				0.326**	-0.247**
SSC				ns	0.241**
TA					-0.841**

**: Statistical significance at P ≤ 0.01; ns: not significant; SSC, soluble solids content; TA, titratable acidity; RI, ripening index; TPC, total phenolics content; TFC, total flavonoids content; AsA, Ascorbic acid; RAC, relative antioxidant content.

Table 4. Pearson's correlation coefficients observed between climatological traits.

	pre	tmx	tmn	tmd	trg	rh	rad
pre_tot	0.085	-0.296**	-0.127	-0.281**	-0.399***	0.591***	-0.772***
pre		-0.127	0.007	-0.076	-0.251**	0.408***	-0.030
tmx			0.873***	0.979***	0.780***	-0.739***	0.678***
tmn				0.948***	0.376***	-0.454***	0.582***
tmd					0.645***	-0.664***	0.699***
trg						-0.822***	0.541***
rh							-0.777***

Statistical significance at **: P ≤ 0.01; ***: P ≤ 0.001; Total precipitation, pre_tot; precipitation, pre; average maximum daily temperature, tmx; average minimum daily temperature, tmn; average daily temperature, tmd; mean daily temperature range, trg; mean daily relative humidity, rh; solar radiation, rad.

Table 5. Mean values of the mixed-effect model coefficients of the traits studied for each cluster for the 155 accessions.

Clusters	**1**	**2**	**3**	**4**	**5**
AsA (int)	0.492	0.244	0.283	-0.568	-0.056
AsA (pre)	-0.044	-0.060	-0.037	0.396	0.126
AsA (rad)	0.694	0.688	0.780	0.880	0.811
AsA (trg)	-0.561	-0.495	-0.495	-0.325	-0.442
RAC (int)	0.398	0.466	0.932	-0.582	-0.332
RAC (rad)	0.051	0.277	0.129	-0.144	-0.158
RAC (tmn)	0.156	-0.190	0.080	-0.082	0.079
RI (int)	1.133	0.962	-0.953	-0.931	0.102
RI (pre)	-0.064	-0.033	-0.365	-0.276	-0.131
RI (rad)	-0.200	-0.182	-0.024	-0.055	-0.243
SSC (int)	0.943	-0.483	0.346	-0.239	0.031
SSC (rad)	-0.243	0.092	-0.015	0.167	-0.132
SSC (tmn)	0.006	-0.098	-0.002	-0.114	0.082
TA (int)	1.064	0.893	-1.022	-1.000	0.033
TA (rad)	0.156	0.098	-0.025	0.015	0.053
TA (tmn)	-0.017	-0.002	0.439	0.346	0.142
TFC (int)	0.546	0.533	0.508	-0.598	-0.313
TFC (pre)	-0.049	-0.033	-0.094	0.196	0.000
TFC (rad)	0.458	0.600	0.475	-0.098	0.077
TFC (tmn)	-0.236	-0.467	-0.313	-0.505	-0.321
TPC (int)	0.743	0.671	0.790	-0.908	-0.362
TPC (rad)	0.700	0.658	0.671	0.344	0.422
TPC (tmn)	-0.117	-0.285	-0.202	-0.070	-0.151
TPC (trg)	0.218	0.156	0.225	0.304	0.309

Intercept, int; minimum temperature, tmn; temperature range, trg; precipitation, pre; solar radiation, rad; soluble solids, SSC; titrable acidity, TA; ripening index, RI; total phenolic content, TPC; total flavonoid content, TFC; relative antioxidant content,RAC; ascorbic acid, AsA.

Table 6. Mean values of the mixed-effect model coefficients of the traits studied according to foreign or local accession.

Clusters	**Foreign**	**Local**
TPC (int)	-0.624	0.353
TPC (rad)	0.442	0.558
TPC (tmn)	-0.118	-0.189
TPC (trg)	0.235	0.266
TFC (int)	-0.356	0.201
TFC (pre)	0.133	-0.045
TFC (rad)	0.043	0.352
TFC (tmn)	-0.432	-0.352
RAC (int)	-0.464	0.280
RAC (rad)	-0.170	0.088
RAC (tmn)	≤ 0.001	-0.007
AsA (int)	-0.353	0.200
AsA (pre)	0.260	0.020
AsA (rad)	0.855	0.741
AsA (trg)	-0.403	-0.473
SSC (int)	-0.002	0.015
SSC (rad)	0.017	-0.040
SSC (tmn)	-0.036	-0.008
TA (int)	-0.339	0.191
TA (rad)	0.065	0.017
TA (tmn)	0.229	0.135
RI (int)	-0.270	0.260
RI (pre)	-0.188	-0.143
RI (rad)	-0.159	-0.160

Intercept, int; minimum temperature, tmn; temperature range, trg; precipitation, pre; solar radiation, rad; soluble solids, SSC; titrable acidity, TA; ripening index, RI; total phenolic content, TPC; total flavonoid content, TFC; relative antioxidant content,RAC; ascorbic acid, AsA.

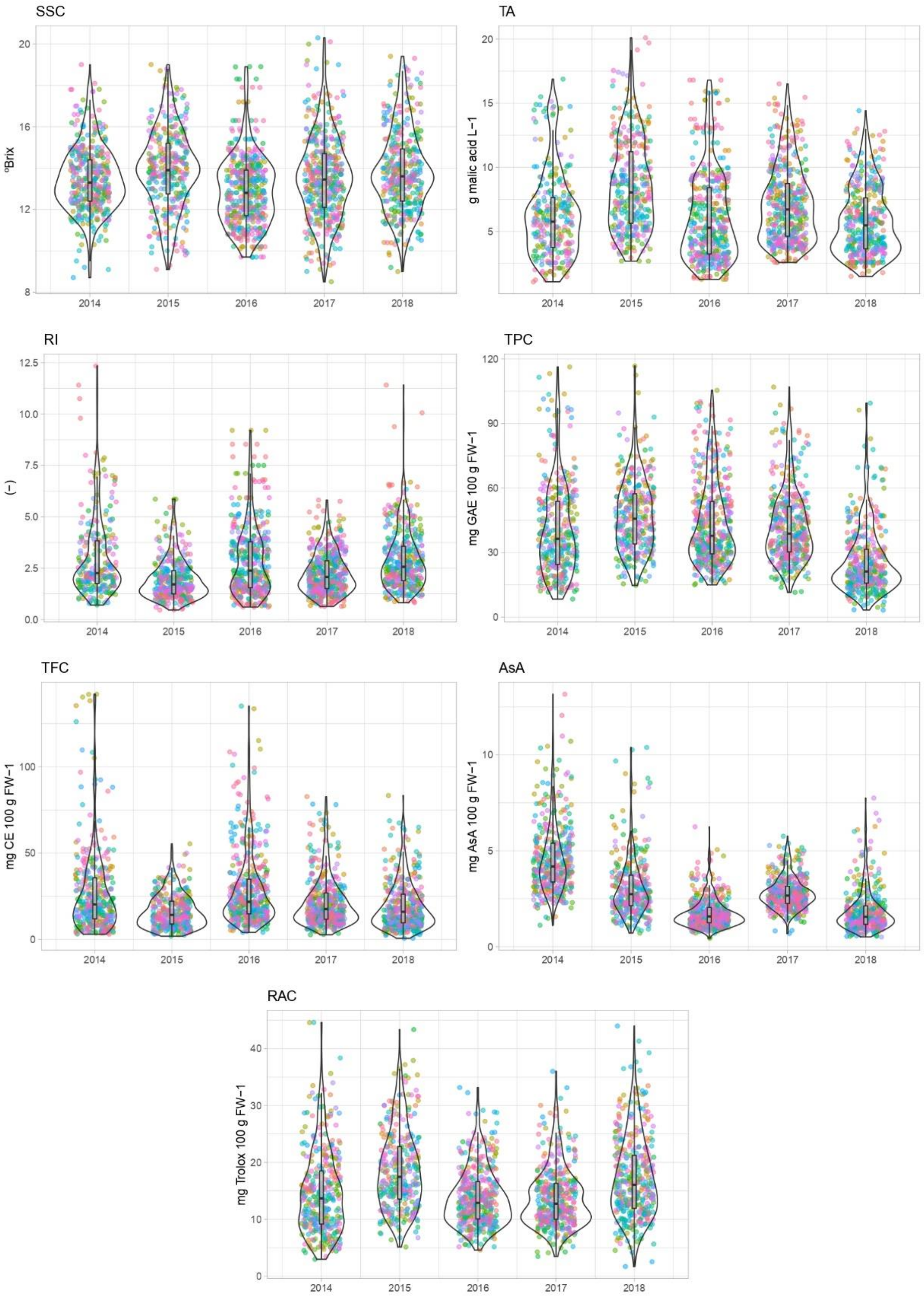

Figure 1. Violin plots showing the variability between accessions (dots) and years of fruit quality parameters, computed between June 1st each year and the harvest day. Abbreviations: soluble solids, SSC; titrable acidity, TA; ripening index, RI; total phenolic content, TPC; total flavonoid content, TFC; relative antioxidant content,RAC; ascorbic acid, AsA.

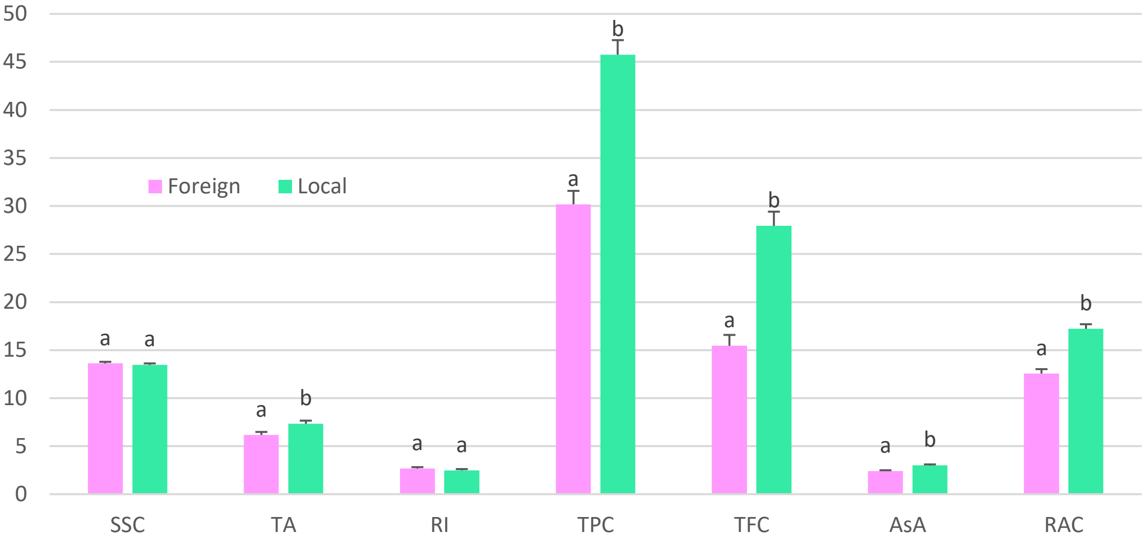

Figure 2. Mean for the different basic quality traits and biochemical compounds studied according to foreign or local accession. Vertical bars indicate ±SE. Different letters indicate significant differences (p≤0.05). Abbreviations: soluble solids, SSC; titrable acidity, TA; ripening index, RI; total phenolic content, TPC; total flavonoid content, TFC; relative antioxidant content,RAC; ascorbic acid, AsA.

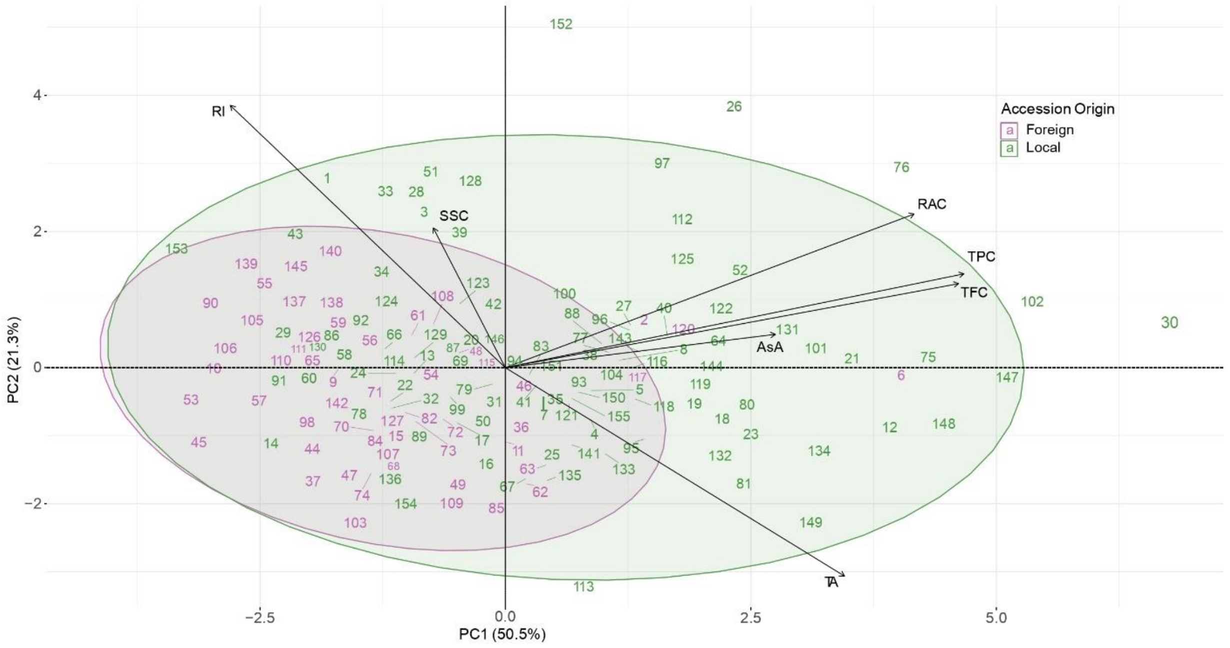

Figure 3. Principal components analysis (PCA) for the basic fruit quality and biochemical antioxidant traits evaluated on the 155 apple accessions. Analysis was performed using mean data of the 5 years of study (2014-2018). Abbreviations: SSC, soluble solids content; TA, titrable acidity; RI, ripening index; TPC, total phenolics content; TFC, total flavonoids content; RAC, relative antioxidant content; AsA, ascorbic acid.

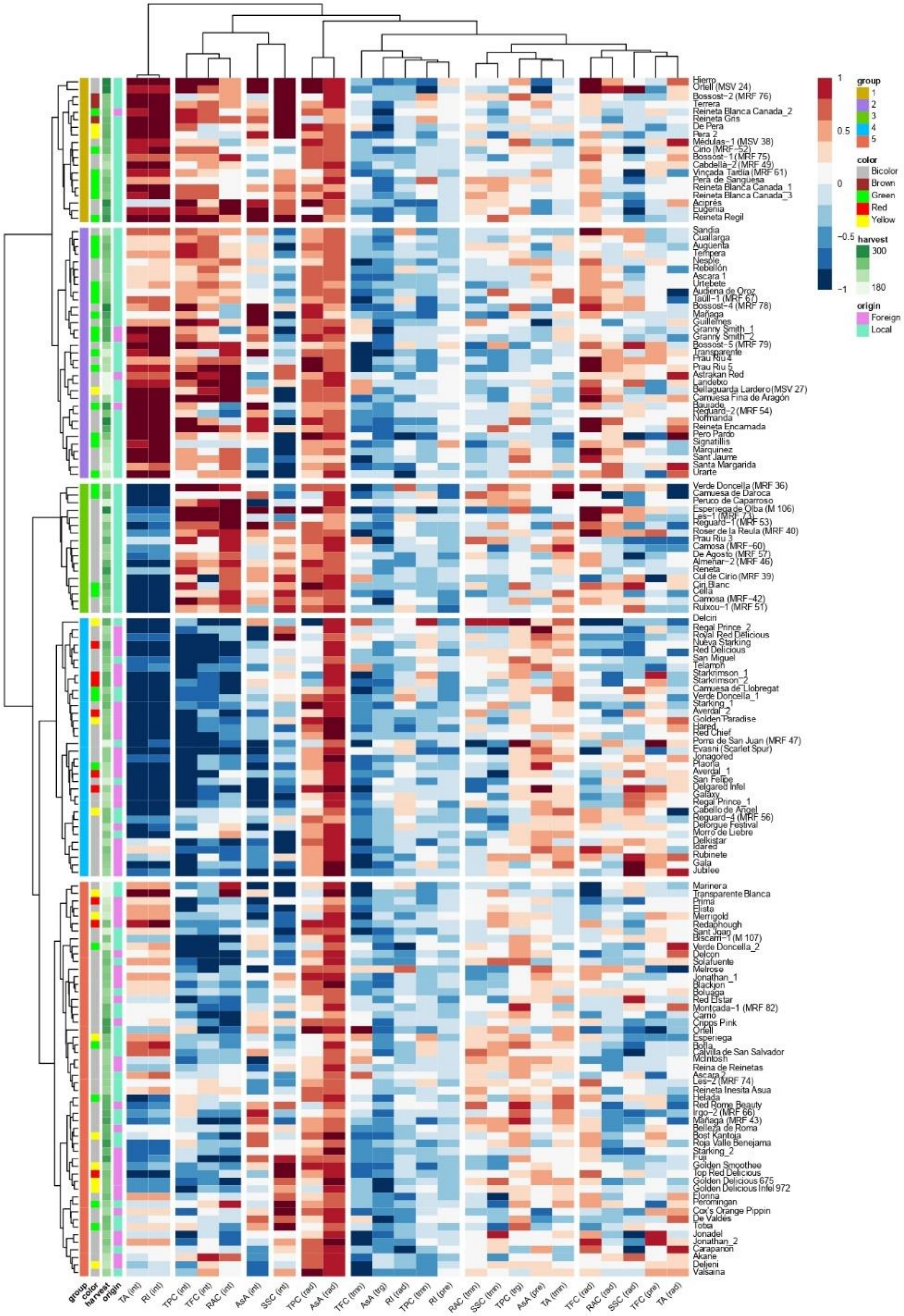
Hierro
Ortell (MSV 24)
Bossost−2 (MRF 76)
Terrera
Reineta Blanca Canada_2
Reineta Gris
De Pera
Pera 2
Médulas−1 (MSV 38)
Cirio (MRF−52)
Bossost−1 (MRF 75)
Cabdellà−2 (MRF 49)
Vinçada Tardia (MRF 61)
Pera de Sangüesa
Reineta Blanca Canada_1
Reineta Blanca Canada_3
Aciprés
Eugenia
Reineta Regil
Sandia
Cuallarga
Augüenta
Tempera
Nesple
Rebellón
Ascara 1
Urtebete
Audiena de Oroz
Taüll−1 (MRF 67)
Bossost−4 (MRF 78)
Mañaga
Guillemes
Granny Smith_1
Granny Smith_2
Bossost−5 (MRF 79)
Transparente
Prau Riu 4
Prau Riu 5
Astrakan Red
Landetxo
Bellaguarda Lardero (MSV 27)
Camuesa Fina de Aragón
Baujade
Reguard−2 (MRF 54)
Normanda
Reineta Encarnada
Pero Pardo
Signatillis
Marquinez
Sant Jaume
Santa Margarida
Urarte
Verde Doncella (MRF 36)
Camuesa de Daroca
Peruco de Caparroso
Esperiega de Olba (M 106)
Les−1 (MRF 73)
Reguard−1 (MRF 53)
Roser de la Reula (MRF 40)
Prau Riu 3
Camosa (MRF−60)
De Agosto (MRF 57)
Almenar−2 (MRF 46)
Reneta
Cul de Cirio (MRF 39)
Ciri Blanc
Cella
Camosa (MRF−42)
Ruixou−1 (MRF 51)
Delciri
Regal Prince_2
Royal Red Delicious
Nueva Starking
Red Delicious
San Miguel
Telamon
Starkrimson_1
Starkrimson_2
Camuesa de Llobregat
Verde Doncella_1
Starking_1
Averdal_2
Golden Paradise
Hared
Red Chief
Poma de San Juan (MRF 47)
Evasni (Scarlet Spur)
Jonagored
Plaona
Averdal_1
San Felipe
Delgared Infel
Galaxy
Regal Prince_1
Cabello de Ángel
Reguard−4 (MRF 56)
Delorgue Festival
Morro de Liebre
Delkistar
Idared
Rubinete
Gala
Jubilee
Marinera
Transparente Blanca
Prima
Elista
Merrigold
Redaphough
Sant Joan
Biscarri−1 (M 107)
Verde Doncella_2
Delcon
Solafuente
Melrose
Jonathan_1
Blackjon
Boluaga
Red Elstar
Montçada−1 (MRF 82)
Carrió
Cripps Pink
Ortell
Esperiega
Bofla
Calvilla de San Salvador
McIntosh
Reina de Reinetas
Ascara 2
Les−2 (MRF 74)
Reineta Inesita Asua
Helada
Red Rome Beauty
Irgo−2 (MRF 66)
Mañaga (MRF 43)
Belleza de Roma
Bost Kantoia
Roja Valle Benejama
Starking_2
Fuji
Golden Smoothee
Top Red Delicious
Golden Delicious 675
Golden Delicious Infel 972
Elonna
Peromingan
Cox's Orange Pippin
De Valdés
Totxa
Jonadel
Jonathan_2
Carapanón
Akane
Deljeni
Valsaina
group
color
harvest
origin
TA (int)
RI (int)
TPC (int)
TFC (int)
RAC (int)
AsA (int)
SSC (int)
TPC (rad)
AsA (rad)
TFC (tmn)
AsA (trg)
RI (rad)
TPC (tmn)
RI (pre)
RAC (tmn)
SSC (tmn)
TPC (trg)
AsA (pre)
TA (tmn)
TFC (rad)
RAC (rad)
SSC (rad)
TFC (pre)
TA (rad)
1
0.5
0
−0.5
−1
group
1
2
3
4
5
color
Bicolor
Brown
Green
Red
Yellow
harvest
300
180
origin
Foreign
Local

Figure 4. Two-way hierarchical analysis of the 155 accessions based on the Euclidean distance on the model coefficients space. Apple accessions are grouped into five groups according to the dendrogram. Positive and negative coefficients indicate the intercept (int) and the effect of climate variables (minimum temperature, tmn; temperature range, trg; precipitation, pre; radiation, rad) on different biochemical traits (SSC, soluble solids content; TA, titrable acidity; RI, ripening index; TPC, total phenolics content; TFC, total flavonoids content; RAC, relative antioxidant content; AsA, ascorbic acid). Additionally, values of auxiliary variables not used in the cluster analysis are also shown: skin color, harvest date (in julian days), and origin.

Supplementary files

Supplementary File 1. Violin plots showing the variability between accessions (dots) and years of the harvest date and the climate parameters, computed between June 1st each year and the harvest day.

Supplementary File 2. ANOVA results for the effect of accession and year on the seven traits studied for the average of the five years of study.

Supplementary File 3. Mean for all years of study 2014-2018 of the 155 apple accessions for the seven different traits studied.

Supplementary File 4. Eigenvalues of the principal components analysis, and variance explained by each component.

Supplementary File 5. Eigenvectors and accumulative variance of the 5 principal components (PCs).

Supplementary File 1. Violin plots showing the variability between accessions (dots) and years of the harvest date and the climate parameters, computed between June 1st each year and the harvest day.

Supplementary File 2. ANOVA results for the effect of accession and year on the seven traits studied for the average of the five years of study.

Source of variation	**Units**	**Accession (A)**	**Year (Y)**	**A x Y**
SSC	°Brix	***	***	***
TA	g malic acid L-1	***	***	***
RI	-	***	***	***
TPC	mg GAE 100 g FW-1	***	***	***
TFC	mg CE 100 g FW-1	***	***	***
AsA	mg AsA 100 g FW-1	***	***	***
RAC	mg Trolox 100 g FW-1	***	***	***

Data were evaluated by two-way variance (ANOVA); *** $P \leq 0.001$.

Supplementary File 3. Mean for all years of study (2014-2018) of the 155 apple accessions for the seven different traits studied.

Variety	Nº	SSC	TA	RI	TPC	TFC	AsA	RAC	Class.
Aciprés	1	16.1	2.8	5.9	35.1	16.6	2.4	18.0	Local
Akane	2	14.1	7.1	2.0	55.6	34.7	2.7	21.4	Foreign
Almenar-2 (MRF 46)	3	14.0	3.0	5.2	39.8	25.6	2.6	19.8	Local
Ascara 1	4	12.0	7.6	1.5	49.9	31.1	2.7	15.0	Local
Ascara 2	5	13.2	8.0	1.7	47.1	29.1	2.7	17.4	Local
Astrakan Red	6	12.3	12.2	1.1	74.2	55.0	2.7	26.4	Foreign
Audiena de Oroz	7	12.8	7.4	1.9	46.4	25.9	2.2	16.5	Local
Augüenta	8	12.8	6.9	1.9	51.4	32.5	2.8	19.2	Local
Averdal_1	9	12.3	4.0	3.3	27.4	6.7	2.8	13.2	Foreign
Averdal_2	10	12.9	3.1	4.1	17.7	13.1	2.0	7.8	Foreign
Baujade	11	14.0	9.5	1.6	34.5	17.2	3.0	13.5	Foreign
Bellaguarda Lardero (MSV 27)	12	10.1	11.6	0.9	61.9	43.9	4.6	22.5	Local
Belleza de Roma	13	14.3	5.9	2.5	31.8	17.0	2.5	13.5	Local
Biscarri-1 (M 107)	14	12.8	5.0	2.7	15.2	6.0	3.4	6.5	Local
Blackjon	15	13.6	7.4	1.9	27.9	16.4	2.5	10.7	Foreign
Bofla	16	13.2	9.1	1.5	34.9	19.4	2.8	12.2	Local
Boluaga	17	14.1	7.9	1.8	34.1	16.4	2.8	12.5	Local
Bossost-1 (MRF 75)	18	12.6	10.7	1.3	57.9	35.7	3.0	20.4	Local
Bossost-2 (MRF 76)	19	17.1	14.0	1.3	53.1	31.1	2.4	19.5	Local
Bossost-4 (MRF 78)	20	15.1	6.1	2.5	37.5	17.0	4.0	12.8	Local
Bossost-5 (MRF 79)	21	13.4	10.5	1.3	72.9	58.7	3.4	20.9	Local
Bost Kantoia	22	13.1	5.5	2.5	29.0	12.6	3.2	10.9	Local
Cabdellà-2 (MRF 49)	23	13.9	12.6	1.1	53.3	34.5	4.6	17.4	Local
Cabello de Angel	24	12.2	4.4	2.9	34.8	17.3	2.3	13.3	Local
Calvilla de San Salvador	25	12.6	8.9	1.4	33.0	18.0	4.8	13.4	Local
Camosa (MRF-42)	26	15.5	3.3	4.7	72.3	46.5	4.5	25.0	Local
Camosa (MRF-60)	27	12.5	5.9	2.4	46.8	30.9	3.7	21.5	Local
Camuesa de Daroca	28	12.5	2.5	5.9	40.4	21.6	3.2	20.1	Local
Camuesa de Llobregat	29	14.4	3.9	4.0	28.8	9.8	2.6	9.2	Local
Camuesa Fina de Aragón	30	10.6	12.3	0.9	98.1	89.0	3.4	29.5	Local
Carapanón	31	13.4	7.8	1.8	39.2	24.2	2.5	14.3	Local
Carrió	32	13.0	5.6	2.3	26.5	15.2	2.7	12.1	Local
Cella	33	14.8	2.9	5.5	40.0	22.7	2.4	19.0	Local
Ciri Blanc	34	13.4	3.3	4.1	38.7	15.2	2.2	17.9	Local
Cirio (MRF-52)	35	14.7	8.8	1.8	44.9	27.7	2.1	16.1	Local
Cox's Orange Pippin	36	14.8	9.1	1.7	33.1	17.2	4.3	12.1	Foreign
Cripps Pink	37	12.7	6.8	1.9	20.0	9.1	2.1	8.7	Foreign
Cuallarga	38	13.4	6.9	2.0	48.9	33.2	2.2	17.8	Local
Cul de Cirio (MRF 39)	39	14.4	3.3	4.5	40.8	15.4	3.1	21.2	Local
De Agosto (MRF 57)	40	12.7	6.9	2.0	54.8	32.3	3.2	22.2	Local
De Pera	41	17.0	10.5	1.7	40.9	19.7	2.7	15.7	Local
De Valdés	42	14.3	5.0	3.1	38.1	21.7	4.1	14.4	Local
Delciri	43	12.9	2.1	6.2	30.5	13.8	2.8	12.8	Local
Delcon	44	13.0	6.5	2.2	20.9	8.8	2.4	9.3	Foreign
Delgared Infel	45	12.6	4.4	2.9	18.6	7.8	1.4	5.9	Foreign
Deljeni	46	13.6	6.7	2.1	41.9	23.4	2.7	15.6	Foreign
Delkistar	47	12.3	6.6	1.9	23.3	8.4	2.7	9.6	Foreign
Delorgue Festival	48	12.7	4.7	2.7	41.4	23.4	2.2	15.3	Foreign
Elista	49	12.2	9.2	1.4	28.9	13.9	2.1	12.6	Foreign
Esperiega	50	13.4	8.5	1.7	37.9	20.8	2.3	13.9	Local
Esperiega de Olba (M 106)	51	14.2	2.8	5.6	43.9	21.7	3.1	20.6	Local
Eugenia	52	17.0	8.8	2.0	54.8	37.4	4.2	25.6	Local
Evasni (Scarlet Spur)	53	12.5	3.5	3.8	18.5	6.9	1.7	7.1	Foreign
Florina	54	14.2	6.9	2.4	30.5	18.4	2.4	14.2	Foreign
Fuji	55	16.9	4.2	4.1	25.5	9.9	2.7	10.5	Foreign

Gala	56	13.6	4.8	3.2	29.6	16.8	2.4	13.0	Foreign
Galaxy	57	12.2	3.9	3.2	21.7	8.2	1.7	11.2	Foreign
Golden Delicious 675	58	15.2	5.1	3.2	25.2	11.8	2.9	10.5	Local
Golden Delicious Infel 972	59	15.0	4.7	3.3	24.3	16.7	2.7	12.7	Foreign
Golden Paradise	60	13.5	4.2	3.5	25.5	10.7	2.5	9.3	Local
Golden Smoothee	61	15.1	4.9	3.1	33.1	15.4	3.9	11.2	Foreign
Granny Smith_1	62	12.3	10.3	1.3	34.4	20.3	2.8	13.0	Foreign
Granny Smith_2	63	12.5	10.0	1.4	37.1	20.6	3.1	14.1	Foreign
Guillemes	64	12.6	6.5	2.0	57.4	36.3	4.7	18.0	Local
Hared	65	12.5	4.0	3.5	25.9	10.1	2.4	13.5	Foreign
Helada	66	12.9	4.2	3.3	34.3	16.1	2.4	13.6	Local
Hierro	67	14.5	11.9	1.4	29.0	13.7	4.1	11.5	Local
Idared	68	12.2	7.5	1.8	26.7	12.1	1.9	11.6	Foreign
Irgo-2 (MRF 66)	69	13.2	4.7	2.9	35.2	17.0	3.7	14.0	Local
Jonadel	70	13.8	6.7	2.1	25.9	11.0	2.9	9.8	Foreign
Jonagored	71	13.5	6.1	2.5	28.0	12.2	2.2	12.5	Foreign
Jonathan_1	72	14.1	7.5	1.9	30.5	19.0	2.9	11.5	Foreign
Jonathan_2	73	14.8	7.9	1.9	29.1	13.6	2.8	11.3	Foreign
Jubilee	74	12.3	7.2	1.8	25.4	10.4	2.2	10.1	Foreign
Landetxo	75	12.9	12.2	1.1	70.5	51.1	3.6	26.3	Local
Les-1 (MRF 73)	76	13.0	4.3	3.0	84.6	72.8	3.0	30.8	Local
Les-2 (MRF 74)	77	13.4	6.9	2.0	49.2	38.7	2.5	18.0	Local
Magaña (MRF 43)	78	12.2	4.6	2.8	26.3	11.9	3.2	10.2	Local
Mañaga	79	12.5	5.5	2.5	30.5	16.1	5.2	12.5	Local
Marinera	80	10.7	8.8	1.3	61.5	46.1	2.1	20.7	Local
Marquinez	81	10.2	11.7	0.9	54.1	40.2	2.4	19.0	Local
McIntosh	82	13.8	6.8	2.1	31.3	17.6	1.9	12.6	Foreign
Médulas-1 (MSV 38)	83	14.4	7.5	2.1	41.5	22.7	3.3	15.1	Local
Melrose	84	12.9	6.7	2.0	26.5	15.3	2.0	11.2	Foreign
Merrigold	85	10.3	9.3	1.3	34.8	17.9	2.1	14.9	Foreign
Montcada-1 (MRF 82)	86	14.6	4.7	3.4	29.7	14.3	2.0	12.8	Local
Morro de Liebre	87	11.6	4.0	3.0	39.8	19.5	2.7	16.4	Local
Nesple	88	13.5	6.7	2.1	52.9	31.9	2.5	19.0	Local
Normanda	89	13.1	7.2	2.3	28.4	12.2	3.1	11.7	Local
Nueva Starking	90	14.1	3.0	4.9	20.8	9.4	1.7	9.8	Foreign
Ortell	91	13.8	4.7	3.3	25.1	8.4	2.3	10.1	Local
Ortell (MSV 24)	92	14.5	4.6	3.5	31.3	14.1	2.6	12.3	Local
Pera 2	93	17.0	10.3	1.6	37.4	20.4	4.3	13.9	Local
Pera de Sangüesa	94	14.9	7.4	2.1	42.4	22.1	2.3	16.0	Local
Pero Pardo	95	12.8	11.4	1.4	45.6	26.9	2.9	18.5	Local
Peromingan	96	14.8	8.0	2.0	47.5	34.8	2.7	21.1	Local
Peruco de Caparroso	97	12.3	2.9	4.6	59.8	47.2	2.2	28.1	Local
Plaona	98	11.7	4.4	2.7	25.5	11.5	1.8	11.4	Foreign
Poma de San Juan (MRF 47)	99	11.3	4.6	2.2	38.0	22.5	1.8	15.4	Local
Prau Riu 3	100	14.8	6.3	2.4	50.2	24.5	2.8	21.7	Local
Prau Riu 4	101	13.3	9.0	1.4	70.0	50.0	2.9	22.0	Local
Prau Riu 5	102	14.1	11.5	1.2	87.0	81.0	3.9	24.9	Local
Prima	103	11.1	7.9	1.4	20.4	8.4	2.0	11.0	Foreign
Rebellón	104	13.6	7.3	1.9	46.8	31.3	3.1	17.4	Local
Red Chief	105	13.2	3.1	4.4	21.6	9.3	2.4	11.2	Foreign
Red Delicious	106	13.7	3.6	4.1	21.7	8.9	1.8	10.6	Foreign
Red Elstar	107	13.3	7.3	1.9	25.7	13.2	2.7	10.6	Foreign
Red Rome Beauty	108	14.2	4.9	3.2	38.1	19.9	2.7	14.7	Foreign
Redaphough	109	12.7	9.6	1.4	29.2	12.3	2.1	11.6	Foreign
Regal Prince_1	110	12.9	3.7	3.6	23.7	9.8	2.0	13.2	Foreign
Regal Prince_2	111	13.3	3.7	3.7	31.0	14.9	2.0	10.6	Foreign
Reguard-1 (MRF 53)	112	15.1	4.8	3.3	50.3	30.0	5.9	24.9	Local
Reguard-2 (MRF 54)	113	13.2	17.3	0.8	40.9	13.6	3.0	11.0	Local
Reguard-4 (MRF 56)	114	13.2	4.5	3.0	35.3	20.7	2.0	13.8	Local
Reina de Reinetas	115	14.0	6.6	2.2	40.2	18.6	2.4	17.4	Foreign

Reineta Blanca Canada_1	116	15.7	10.0	1.6	45.6	30.3	3.3	18.5	Local
Reineta Blanca Canada_2	117	15.3	9.6	1.6	48.0	32.8	3.7	15.7	Foreign
Reineta Blanca Canada_3	118	14.2	9.8	1.5	53.1	31.3	2.9	16.3	Local
Reineta Encarnada	119	14.2	10.1	1.6	56.5	30.7	3.6	18.0	Local
Reineta Gris	120	16.6	9.7	1.7	57.0	32.9	3.6	20.7	Foreign
Reineta Inesita Asua	121	13.5	8.3	1.5	46.7	28.0	2.1	17.2	Local
Reineta Regil	122	17.2	9.7	1.8	49.8	32.9	5.1	18.5	Local
Reneta	123	13.0	4.0	3.4	39.4	22.9	2.3	18.6	Local
Roja Valle Benejama	124	13.0	3.6	3.6	34.7	18.1	2.1	17.2	Local
Roser de la Reula (MRF 40)	125	14.2	5.5	2.7	62.1	34.6	3.5	25.1	Local
Royal Red Delicious	126	15.6	5.4	3.1	26.6	10.2	1.9	13.4	Foreign
Rubinete	127	15.3	7.7	2.2	31.1	13.4	2.1	10.2	Foreign
Ruixou-1 (MRF 51)	128	15.6	3.5	4.8	45.5	27.4	1.8	21.7	Local
San Felipe	129	11.8	3.9	3.1	34.5	19.3	2.6	14.5	Local
San Miguel	130	14.2	4.1	3.4	27.7	13.3	2.3	10.8	Local
Sandia	131	12.6	7.9	1.6	61.9	48.0	4.4	20.9	Local
Sant Jaume	132	11.5	11.9	1.0	53.8	35.3	2.3	19.0	Local
Sant Joan	133	10.4	8.1	1.3	48.9	24.3	3.1	16.1	Local
Santa Margarida	134	10.3	12.2	0.9	66.4	42.9	2.9	23.1	Local
Signatillis	135	11.1	9.0	1.3	42.7	24.0	2.7	12.6	Local
Solafuente	136	12.5	7.3	1.8	24.5	9.9	2.5	10.3	Local
Starking_1	137	14.3	3.8	4.0	24.9	13.9	2.2	13.8	Foreign
Starking_2	138	14.1	3.9	4.0	30.2	14.5	2.3	12.5	Foreign
Starkrimson_1	139	14.2	3.1	5.2	25.0	11.6	2.2	12.8	Foreign
Starkrimson_2	140	14.8	3.3	4.9	28.9	14.0	2.5	14.3	Foreign
Taüll-1 (MRF 67)	141	12.3	9.6	1.3	45.8	23.1	2.0	17.3	Local
Telamon	142	13.0	4.9	2.5	30.0	15.8	2.1	10.4	Foreign
Tempera	143	13.1	7.1	1.9	53.9	34.7	2.9	19.2	Local
Terrera	144	17.8	12.8	1.4	51.1	33.3	3.2	18.2	Local
Top Red Delicious	145	15.6	3.5	4.5	26.1	11.5	1.8	14.2	Foreign
Totxa	146	15.9	6.9	2.4	42.2	20.7	3.5	13.0	Local
Transparente	147	11.7	12.5	0.9	73.1	55.0	5.3	24.2	Local
Transparente Blanca	148	10.4	12.8	0.8	66.9	47.9	4.5	23.6	Local
Urarte	149	10.5	16.2	0.7	62.8	40.4	2.2	21.1	Local
Urtebete	150	12.6	7.0	1.6	47.5	33.0	2.2	17.2	Local
Valsaina	151	14.3	7.8	2.0	45.3	26.3	2.4	16.1	Local
Verde Doncella (MRF 36)	152	13.5	1.8	8.6	78.4	45.9	2.6	20.4	Local
Verde Doncella_1	153	13.4	2.2	6.6	22.2	11.4	1.8	11.9	Local
Verde Doncella_2	154	12.2	10.8	2.3	20.7	9.3	3.1	10.9	Local
Vinçada Tardía (MRF 61)	155	14.2	9.0	1.7	48.7	29.2	2.4	15.3	Local

SSC, soluble solids content; TA, titratable acidity; RI, ripening index; TPC, total phenolics content; TFC, total flavonoids content; AsA, Ascorbic acid; RAC, relative antioxidant content; Class., classification. Units: SSC:ºBrix; TA: g malic acid L^{-1}; TPC: mg GAE 100 g FW^{-1}; TFC: mg CE 100 g FW^{-1}; Ascorbic acid: mg AsA 100 g FW^{-1}; RAC: mg Trolox 100 g FW^{-1}.

Supplementary File 4. Eigenvalues of the principal components analysis, and variance explained by each component.

Principal Component	Eigenvalue	Variance (%)	Cumulative variance (%)
1	3.53	50.5	50.5
2	1.49	21.3	71.8
3	1.08	15.4	87.2
4	0.61	8.7	95.9
5	0.14	1.9	97.8
6	0.12	1.7	99.5
7	0.03	0.5	100

Supplementary File 5. Eigenvectors and accumulative variance of the 5 principal components (PCs).

Variable	PC 1	PC 2	PC 3	PC 4	PC 5
SSC	-0.15	0.41	0.80	0.41	-0.04
TA	0.69	-0.61	0.21	0.18	0.26
RI	-0.56	0.77	-0.14	-0.10	0.25
RAC	0.83	0.45	-0.15	0.08	-0.04
TFC	0.92	0.24	-0.17	0.08	-0.04
TPC	0.93	0.28	-0.13	0.09	0.02
AsA	0.55	0.10	0.55	-0.61	0.00

SSC, soluble solids content; TA, titratable acidity; RI, ripening index; RAC, relative antioxidant content; TFC, flavonoids; TPC, total phenollics content; AsA, ascorbic acid.