**Characterization of composition traits related to organoleptic and functional quality for the differentiation, selection and enhancement of local varieties of tomato from different cultivar groups**

Running title: Composition of local tomato varieties from different cultivar groups

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**Abstract**

Tomato (*Solanum lycopersicum*) local varieties are having an increasing demand. We characterized 69 local tomato accessions from eight cultivar groups for proximate composition traits, major sugars, acids and antioxidants. A large diversity was found, with differences among accessions of almost ten-fold for lycopene. Significant differences were found among cultivar group means for most traits. The Cherry and Penjar groups generally presented higher dry matter, soluble solids content, titratable acidity, taste index, β-carotene, ascorbic acid, total phenolics, and antioxidant activity that the other groups. Wide ranges of variation were found within each cultivar group. Positive correlations were found between proximate traits related to taste and antioxidants. The multivariate principal components analysis confirms the distinct profile of the Cherry and Penjar groups and the large variation within groups. The results will be useful for the differentiation, enhancement and selection of local tomato varieties with improved organoleptic properties and functional quality.

**Keywords:** *Solanum lycopersicum*, chemical composition, local varieties, organoleptic quality, functional quality, cultivar groups, selection

## 1. Introduction

Commercial production of tomato (*Solanum lycopersicum* L.) in developed regions of the world is mostly based on modern varieties, frequently genetically uniform F1 hybrids, which have a high yield, multiple resistance to diseases, and long shelf life (Díez and Nuez, 2008). Despite the clear productive advantages of modern tomato varieties, consumers often complain of their reduced organoleptic quality (Causse et al., 2010). Low quality of many modern tomato varieties has been attributed to a tradeoff between yield and concentration of compounds involved in taste, in particular when cultivated under suboptimal conditions of temperature and illumination, as well as to the pleiotropic effect on organoleptic quality of long-shelf life mutations, like *rin*, present in many commercial hybrids of tomato (Díez and Nuez, 2008). This has increased the demand for local and “heirloom” varieties of tomato, which are associated to a better flavour (“flavour of the past”), to local production (Brugarolas et al., 2009), and in some cases, to an increased content in bioactive compounds compared to standard long shelf life varieties (Vrebalov et al., 2002). In consequence, local tomato varieties often reach market prices much higher than those of standard modern varieties (Cebolla-Cornejo et al., 2007; Brugarolas et al., 2009). This increased demand opens the opportunity for the recovery of local tomato varieties for an expanding market.

Tomato is grown in all tropical, subtropical and temperate regions of the world, and differences in local preferences and agroclimatic conditions, in conjunction with microevolutionary forces have resulted in the accumulation of a large phenotypic diversity in local varieties of tomato, with a wide diversity and array of combinations for fruit size, shape, and colour (Rodríguez-Burruezo et al., 2005; Díez and Nuez, 2008). One of the regions with a greatest diversity in local varieties of tomato is the Mediterranean region, which is considered a secondary center of diversity for this crop (Terzopoulos and Bebeli, 2008; Mazzucato et al., 2008; García-Martínez et al., 2013; Figàs et al., 2015), with many local tomato varieties being locally appreciated. In Europe, some of these traditional varieties, like Marmande, Oxheart or San Marzano among others, have also obtained widespread recognition as high quality varieties and are found in markets throughout the continent (Di Gioia et al., 2010; Casals et al., 2011; Ercolano et al., 2014).

Enhancement of local varieties of vegetables for a more economically productive horticulture can be achieved using characterization, selection and breeding approaches (Hurtado et al., 2014). In this respect, the characterization of composition traits relevant for organoleptic and bioactive properties is of interest, as it is increasingly valued by consumers. This information may allow determining composition characteristics distinctive of specific local cultivar groups, the diversity for the chemical composition within each group, as well as to identify accessions within each cultivar group with enhanced values for the target composition traits.

In tomato, taste is mostly related to the content in sugars, acids and their ratio (Navez et al., 1999; Causse et al., 2010; Siddiqui et al., 2015). The main sugars in tomato are the monosaccharides glucose and fructose, which are usually present at equimolar ratios (Beckles, 2012). Due to high activity of acid invertase, the disaccharide sucrose is not detectable or present at low levels in the cultivated tomato fruit (Beauvoit et al., 2014). Regarding acids, citric acid is the main organic acid acid of the tomato fruit (Fernández-Ruiz et al., 2004; Siddiqui et al., 2015). Malic acid and oxalic acid, which usually rank as the second and third most abundant organic acids of tomato, are present at much lower levels than citric acid (Fernández-Ruiz et al., 2004).

The carotenoid lycopene is the most characteristic antioxidant compound of tomato and is responsible of the red color of the ripe fruit (Siddiqui et al., 2015). Another carotenoid of bioactive relevance present in the tomato fruit is β-carotene, although its levels are normally much lower than those of lycopene (Cortés-Olmos et al., 2014). Lycopene and β-carotene intake has been correlated to a reduced risk of certain types of cancer and cardiovascular diseases (Riccioni, 2009; Keikel et al., 2011).

Ascorbic acid, which has antioxidant as well multiple biological effects beneficial for human health, like antiscorbutic and anticarcinogen properties (Du et al., 2012), is also present at significant levels in the tomato fruit (Cortés-Olmos et al., 2014). Phenolics, in particular chlorogenic acid and quercetin, are also present in the tomato fruit in significant concentrations (Siddiqui et al., 2015). Apart from the antioxidant activity displayed by tomato phenolics, they are increasingly recognized as having important biological properties, including anti-inflammatory, anti-microbial, neuroprotective and cardioprotective effects (Del Río et al., 2013).

We have characterized a collection of local varieties of tomato from the Mediterranean region of València (Spain) for chemical composition traits involved in taste and functional quality. The collection contains accessions from different locally recognized cultivar groups (Figàs et al., 2015). Chemical characterization data will provide relevant information on the diversity for these traits among local tomato varieties, as well as on differences among and within cultivar groups. This information will be of interest for the enhancement and selection of local varieties of tomato.

## 2. Material and Methods

### *2.1. Plant material*

A total of 69 accessions of local varieties of tomato from the region of València, situated in the Mediterranean coast of Spain, were used for the analyses of composition. The local varieties used correspond to eight cultivar groups commonly recognized in the region and present different fruit characteristics (Table 1). These accessions have been previously morphologically characterized using conventional descriptors and the phenomics tool Tomato Analyzer (Figàs et al., 2015). Passport data on each of the accessions used is included as Electronic Supplementary Material - Table S1).

Five plants per accession were grown during the spring-summer season of 2013 in an open field plot in Vila-Real (Region of València, Spain) following the standard horticultural practices used in the area for local varieties of tomato. Plants were distributed following a completely randomized design. Further details of plant cultivation conditions can be consulted elsewhere (Figàs et al., 2015).

### *2.2. Preparation of samples*

Five samples of healthy vine-ripened mature red-ripe stage fruit were used for each accession. Fruits were harvested between June 17 and August 16. Each sample consisted of at least three tomatoes from the second to fourth trusses, with a minimum total weight for sample of 250 g. Fruits were brought to the laboratory and were washed and squeezed using a domestic juice extractor. Two aliquots were obtained; one was used for the immediate determination of ascorbic acid and for proximate traits measurement (dry matter, soluble solids, pH, and titratable acidity), and the other was frozen in liquid $N_2$ and stored at -80ºC until analyzed for the rest of traits.

### *2.3. Analytical methods*

Dry matter was calculated from a 10 ml juice sample as 100×dw/fw, where fw and dw are, respectively, fresh weight and dry weight after drying at 105ºC to constant weight. Soluble solids (SS) were measured refractometrically using a drop of juice using a hand-held refractometer. pH was determined in juice using an automatic pHmeter. Titratable acidity (TA) was determined potentiometrically by titrating a 100 ml diluted (1:5) sample of juice with 0.5 N NaOH to pH 8.1, and expressed as percentage of citric acid. Taste index (TI) was determined according to Navez et al. (1999) from the SS and TA values using the formula TI=TA+(SS/(20×TA)). Glucose and fructose were determined using the D-Fructose/D-Glucose Assay Kit (Megazyme International Ltd., Wicklow, Ireland) according to the manufacturer instructions (Megazyme, 2013). In order to calculate concentrations of glucose and fructose, absorbances were measured at 340 nm (Megazyme, 2013) in a Jenway 6305 (Jenway, Essex, UK) spectrophotometer (Megazyme, 2013). Citric acid was determined using the CI9920 enzymatic kit (BEN S.r.l., Milano, Italy) according to the manufacturer instructions. For lycopene and β-carotene determinations, frozen homogenate was extracted overnight with ethanol:hexane (4:3 v/v) in darkness. Subsequently, the hexane phase was separated and lycopene and β-carotene concentrations were determined from UV/V spectrophotometry absorbance values at 503 nm (lycopene) and 450 nm (β-carotene). Ascorbic acid was determined by potentiometric titration with a Titrino 702 (Metrohm, Herisau, S witzerland) using a Metrohm 6.0420.100 combined Pt selective electrode and a 0.005 M chloramine T as standard. Total phenolics were determined according to the Folin-Ciocalteu method using chlorogenic acid as standard, as indicated in Raigón et al. (2008). Antioxidant activity was estimated using the colourimetric DPPH (1,1-diphenyl-2-picrylhydrazyl) assay as described by Sánchez-Moreno et al. (1998) and expressed as Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalents (TE).

*2.4. Data analysis*

Data were subjected to analyses of variance (ANOVA) using a fixed-effects model for variety. The total sums of squares for each trait was partitioned into the sums of squares for accession and residual effects. The average (pooled) standard error (SE) was obtained for each trait from the corresponding ANOVA. The coefficients of phenotypic variation ($CV_P$) and genotypic variation ($CV_G$) for each trait were estimated from the mean value and the estimates for the phenotypic variance and genotypic variance obtained from the sums of squares of the ANOVA analyses (Wricke and Weber, 1986). Mean values for each accession were used to perform additional ANOVA analyses to detect differences among cultivar group means. In this case, the total sums of squares for each trait was partitioned into the sums of squares between and within groups. Significant differences among cultivar group means were estimated using the Duncan multiple range test. Pearson linear coefficients of correlation (r) were calculated between pairs of traits and significance ($P<0.05$) of correlations was evaluated with the Bonferroni test (Hochberg, 1988). Principal components analysis (PCA) was performed for standardized composition data using pairwise Euclidean distances among accessions means. Eigenvalues and percent of variance accounted for each principal component as well as correlation coefficients between composition traits and principal components were calculated. Statistical and PCA analyses were performed with the Statgraphics Centurion XVI version 16.2.04 (StatPoint Technologies Inc, Warrenton, VA, USA) software package.

## 3. Results

### *3.1. Variation parameters*

The accession effect was highly significant (P<0.001) for all composition traits studied and represented between 37.31% (for citric acid) and 76.21% (for total phenolics) of the total variance (Table 2). For all traits, except pH, fructose, and citric acid, the accession effect accounted for more than 50% of the total sums of squares. The average dry matter content of the collection was of 6.49%, while for soluble solids content it was of 5.70%. The pH had an average value of 4.24 and titratable acidity of 0.46%. As a result of the soluble solids content and titratable acidity values the taste index had an average value of 1.1. Fructose presented slightly higher concentration values than glucose (Table 2). Citric acid concentration values were considerably lower than those of sugars. Regarding antioxidants, the highest average concentration values were obtained for total phenolics (601.5 $mg \cdot kg^{-1}$), followed by ascorbic acid (197.3 $mg \cdot kg^{-1}$), lycopene (36.62 $mg \cdot kg^{-1}$), and finally by β-carotene (9.11 $mg \cdot kg^{-1}$). The average antioxidant activity was of 2.27 mmol $TE \cdot kg^{-1}$. A wide range of variation was found among accession means for composition traits. In this respect, for most traits severalfold differences were observed between the minimum and maximum values (Table 2). The traits with a larger value for the relative range (i.e., maximum/minimum values) were lycopene (9.39-fold) and citric acid (6.27-fold), while those with the lowest relative range were pH (1.14-fold) and taste index (1.51-fold). The lowest values for the coefficients of phenotypic ($CV_P$) and genotypic ($CV_G$) variation were obtained for pH, while the highest values for $CV_P$ and $CV_G$ were those of lycopene (Table 2).

*3.2. Differences among cultivar groups*

When considering the proximate traits, significant differences (P<0.05) were found among cultivar group means, except for pH (Table 3). For all traits, the within groups sums of squares accounted for more than 50% of the variance. The highest average dry matter contents were found for the Cherry group, followed by the Penjar group; both groups presented a significantly higher dry matter than the rest of groups (Table 3). For soluble solids content, the Cherry group was significantly higher than the rest of groups, except Penjar; the latter in turn was significantly higher than Borseta and Pruna. For titratable acidity, Cherry also presented values significantly higher than the rest of varieties; and, Penjar ranked second, with values significantly higher than those of Borseta and Cor. For the taste index, again the highest values were those of the Cherry group, with average values significantly higher than the rest of accessions, except Penjar, which had significantly higher values than Pruna (Table 3). Despite the existence of significant differences among group means for four of the proximate composition traits, considerable variation was found for accession means within each of the groups (Table 3). In this respect, in most cases the ranges of variation for each of the cultivar groups overlap with the other groups for all traits, the exception being the Cherry group which does not overlap with several other groups for dry matter, soluble solids, titratable acidity, and taste index (Table 3).

For the sugars (glucose and fructose) and acid (citric acid) measured, significant differences among cultivar group means were found only for fructose (Table 3). As occurred for proximate traits the within groups sums of squares accounted for more than 50% of the variance in all cases. For fructose, the highest values were found for the Cor and Borseta, which were significantly higher than those of Cherry, Pruna, and Penjar. Also, Redona presented values significantly higher than those of Cherry (Table 3). As occurred for proximate traits, considerable variation was found within each of the groups (Table 3). The ranges of variation for each of the cultivar groups overlap with the other groups for all traits, with the exception of the Cherry group, for fructose, does not overlap with the range of variation of Borseta and Cor groups (Table 3).

All antioxidants, as well as the antioxidant activity, displayed significant differences among cultivar group means (Table 3). For β-carotene, ascorbic acid and total phenolics the between groups sums of squares accounted for more than 50% of the variance. The Cherry group presented the highest average values for all antioxidant compounds, (Table 3). The group Penjar ranked second for β-carotene, ascorbic acid, total phenolics and antioxidant activity; however it was the cultivar group with lowest content in lycopene (Table 3). For the rest of groups, few significant differences were found among groups. As for the proximate composition traits, sugars and citric acid, large ranges of variation were found within the cultivar groups, and all the groups overlapped in the range of variation, with the exception of the Cherry group, which only overlapped in the range of variation with the Penjar group for all traits but lycopene (Table 3).

*3.3. Correlations among traits*

A total of 26 linear correlations were significant according to the Bonferroni test at a significance level of $P \leq 0.05$ (Table 4). All the significant correlations detected were positive. Dry matter, soluble solids and titratable acidity were significantly intercorrelated; dry matter and soluble solids were also significantly correlated with taste index. Dry matter, soluble solids and titratable acidity were also significantly correlated with all antioxidant traits, except lycopene (Table 4). Taste index was significantly correlated with ascorbic acid, total phenolics and antioxidant activity. No significant correlations were found involving pH, sugars, citric acid or lycopene.

Antioxidants β-carotene, ascorbic acid and total phenolics, as well as the antioxidant activity, were significantly intercorrelated (Table 4).

### *3.4. Principal components analysis*

The first and second components of the PCA accounted, respectively for 43.9% and 11.6 % of the total variation among accession means (Table 5). The first component was positively correlated with dry matter, soluble solids, titratable acidity, as well as with all antioxidant traits, except lycopene (Table 5). The second principal component was positively correlated with pH, ascorbic acid and antioxidant activity, and negatively with soluble solids, taste index, glucose, fructose, citric acid and lycopene (Table 5). The centroid values for Borseta, Cor, Plana, Pruna, Redona and Valenciana groups cluster together in the PCA plot, with negative values for the first component and intermediate values for the second component. Groups Penjar and Cherry present positive values for the first component and higher (Penjar) and lower (Cherry) values than the rest of cultivar group centroids for the second component (Fig. 1). The projection of the individual accessions in the PCA plot shows that accessions, with the exception of the Cherry group, accessions of the different groups are intermingled (Fig. 1). However, the two most representative groups (Valenciana and Penjar) present a low degree of overlap. The first component separates the accessions of the Cherry group and part of the Penjar group accessions, which plot in the right part of the PCA plot (with first component values above 2), from the rest of accessions (Fig. 1). For the second component, in general for each of the groups there is a broad dispersion. However, for the Penjar and Borseta groups there are no accessions with highly negative values;

conversely, for the Cor group there is only one accession having a positive (although low) value (Fig. 1).

## 4. Discussion

The large diversity for composition traits relevant for taste and functional quality found in the collection of traditional varieties of tomato evaluated matches the wide morphological variation found in the same set of accessions (Figàs et al., 2015). Our data are in agreement with other studies on the diversity for chemical composition traits of local varieties of tomato (Rodríguez-Burruezo et al., 2005; Labate et al., 2011; Panthee et al., 2013; Cortés-Olmos et al., 2014) and reveal that local varieties are highly variable for composition traits and, therefore, amenable to selection.

The average levels for the traits evaluated are generally comparable to other tomato studies (Fernández-Ruiz et al., 2004; Rodríguez-Burruezo et al., 2005; Ilahy et al., 2011; Labate et al., 2011; Panthee et al., 2013; Cortés-Olmos et al., 2014). The somewhat larger values for total phenolics obtained by us compared to other works (Ilahy et al., 2011; Cortés-Olmos et al., 2014) is probably due to the fact that we expressed the results in equivalents of chlorogenic acid, which is the major phenolic compound of tomato (Siddiqui et al., 2015), instead of the commonly used gallic acid (Ilahy et al., 2011) or caffeic acid (Cortés-Olmos et al., 2014). Chlorogenic acid has an antioxidant activity similar to caffeic acid (Rice-Evans et al., 1997), but it has a higher molecular weight, and this may explain the higher values obtained by us. Remarkably, the average taste index, which is related to the relationship between soluble solids content and titratable acidity, had a value above 1, indicating that most of the local varieties evaluated may be considered as tasty (Navez et al., 1999). Probably, the fact that plants used for the present study were cultivated outdoors during the summer season, under conditions that favour the production of tomato fruits with good quality, has contributed to achieving good values for the taste index (Cebolla-Cornejo et al., 2011). With the exception of pH, differences of several-fold have been found for all traits studied. Even in the case of pH, the differences (0.56 units) can be considered as large, as a difference of 1 unit in pH represents 10 times in the concentration of $H^+$ ions. The traits with largest phenotypic and genotypic variation were the contents in lycopene and β-carotene, suggesting that important genetic advances can be achieved by selection in the collection studied (Panthee et al., 2013). Apart from its interest for its functional and bioactive properties (Roccioni et al., 2009; Keikel et al., 2011), both carotenoids are determinant for the fruit color (Siddiqui et al., 2015). In particular high levels of lycopene are associated to intense red fruits (Hyman et al., 2004), and selection of local accessions with high content in lycopene would result in an added value (Ilahy et al., 2011).

The Cherry and Penjar cultivar groups had an average composition profile different from the rest of cultivar groups, with generally higher levels of dry matter, soluble solids, titratable acidity, taste index, β-carotene, ascorbic acid, total phenolics and antioxidant activity. It is well known that Cherry tomatoes normally are tastier than standard regular size tomatoes (Zanor et al., 2009). The fact that the fruit size and yield per plant in Cherry tomatoes is lower than for regular size tomato varieties is probably a main reason for the higher concentration values for most traits observed in this cultivar group (Panthee et al., 2013). Regarding the Penjar tomatoes, this cultivar group is characterized by the presence of the *alc* mutation (Casals et al., 2012; Bota et al., 2014), which interferes with ripening and confers a long shelf life (Vrebalov et al., 2002; Bota et al., 2014). Penjar fruits, on average, have a smaller fruit size than other groups of local varieties, like Borseta, Cor, Plana, Redona and Valenciana (Figàs et al., 2015). Our results support previous observations indicating that the *alc* mutation, apart from delaying ripening, has pleiotropic effects on the physiology of the plant and also on the fruit composition (Vrebalov et al., 2002). A negative effect of the *alc* mutation in composition is a reduction in the concentration of lycopene (Vrebalov et al., 2002). On the other hand, the high antioxidant activity of Penjar tomatoes may play a role in its extended shelf life.

An important diversity has been detected for the traits studied within each of the cultivar groups. Other studies involving cultivar groups of local tomato varieties have found similar results (Cortés-Olmos et al., 2014). In this way, with the exception of the Cherry group, a complete overlap has been found among cultivar groups for all traits. This indicates that the selection of accessions of each cultivar group with an improved content in compounds relevant for taste and functional quality is feasible. This is of great relevance for the enhancement of local varieties associated to high standards of quality (Hurtado et al., 2014), as well as to identify sources of variation for breeding (Rodríguez-Burruezo et al., 2005; Cortés-Olmos et al., 2014). In this respect, genotype × environment (G×E) interactions may be important for the traits studied (Cebolla-Cornejo et al., 2011; Panthee et al., 2013) and further work should be undertaken to evaluate the extent of G×E interactions in this collection.

Positive intercorrelations found between dry matter, soluble solids, titratable acidity, and taste index were expected, as these traits are interrelated (Labate et al., 2011; Panthee et al., 2013). However, positive correlations were also found with these most of these traits and β-carotene, ascorbic acid, and total phenolics. This is relevant as it indicates that, in the collection studied, selection of accessions with high values for traits related to improved taste will also have high content in bioactive compounds and antioxidant activity. The different antioxidants, with the exception of lycopene, and the antioxidant activity were also positively intercorrelated. Ascorbic acid and total phenolics presented the highest correlation values with the antioxidant activity. In this respect, among the antioxidants the highest concentration was found for total phenolics, being followed by ascorbic acid (3-fold less on average). Given that the antioxidant activities of the major tomato phenolics chlorogenic acid and quercetin is higher than that of ascorbic acid (Rice-Evans et al., 1997) and the levels of carotenoids are comparatively much lower than those of phenolics and ascorbic acid, our results support the view that the major contributors to antioxidant activity in tomato are phenolics (Toor and Savage, 2005).

The multivariate PCA analysis confirmed the results obtained with univariate methods and provided a good separation of the Cherry tomato accessions and most of the Penjar group accessions from the rest of varietal groups, which are intermingled and plot in the same area of the PCA graph. This is in contrast with morphological data, which provide a clear separation of the cultivar groups studied here (Figàs et al., 2015). In this respect, cultivar groups of tomato usually are established by morphological traits rather than for composition traits (Díez and Nuez, 2008). The results also reveal that the Penjar and Valenciana cultivar groups, which are the most characteristic ones in the region of València (Figàs et al., 2015), are clearly separated and have a low degree of overlap in the PCA plot, indicating that both groups have different composition profiles, which is important for marketing strategies (Oltman et al., 2014). In this respect, these two types have different uses, with Valenciana being commonly used in salads, while the fruits of Penjar are mostly used for rubbing onto bread (Casals et al., 2012; Bota et al., 2014). The PCA analysis also reveals that there is a large variation in composition profile among Penjar tomato accessions, which present a wide range of values for the first component. This is probably associated to the fact that the Penjar tomato is a conglomerate of accessions carrying the *alc* mutation introgressed in different genetic backgrounds (Casals et al., 2012), rather than a group of accessions with a common genetic background.

## 5. Conclusions

The collection of local varieties evaluated has been very diverse for composition traits involved in taste and functional properties. Two groups (Cherry and Penjar) have shown composition profiles distinct from the rest of groups, which have presented similar average values for the traits studied. The large diversity found within each of the groups, together with the positive correlation between taste and functional quality traits indicates that there are good prospects for the selection of local varieties of each group with improved composition. Our study is of interest for the enhancement of local varieties of tomato as it provides information on differences among and within cultivar groups for traits related to organoleptic and functional quality. These results will allow the selection of local accessions of tomato with better quality and adapted to the demands of consumers.

## Acknowledgements

This work has been funded by Universitat Politècnica de València. Isabel Andújar is grateful to Universitat Politècnica de València for a post-doctoral contract (PAID-10-14).

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## Figure caption

**Fig. 1.** Similarities based on fruit composition among 69 accessions of local varieties of tomato represented on the two first principal components of PCA. First and second components account for 43.9% and 11.6% of the total variation, respectively. The different cultivar groups are represented by different symbols: Borseta (BOR; filled square), Cherry (CHE; filled circle), Cor (COR; filled triangle), Penjar (PEN; filled rhombus), Plana (PLA; open square), Pruna (PRU; open circle), Redona (RED; open triangle), and Valenciana (VAL; open rhombus). First and second component centroids for each of the cultivar groups are indicated using the group code. The continuous and dashed lines encompass, respectively, accessions of the most characteristic local landraces Penjar and Valenciana.