Fruit composition diversity in land races and modern pepino (*Solanum muricatum*) varieties and wild related species

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ABSTRACT

Pepino (*Solanum muricatum*) fruits from 15 accessions of cultivated pepino as well as six accessions from wild relatives were evaluated for contents in dry matter, protein, β-carotene, chlorophylls and seven minerals. Several-fold differences among accessions were found for most traits. Average values obtained were similar to those of melon and cucumber, but the phenolic contents were much higher. Wild species had significantly higher average contents for all traits vs. the cultivated pepino accessions. And, the comparisons among the cultivated pepino varieties showed that the modern varieties were more uniform in composition, and they possessed significantly lower concentrations of protein, P, K, and Zn than local land races. Most of the significant correlations among composition traits were positive. Our studies show that regular consumption of pepino fruits could make a significant contribution to the recommended daily intake of P, K, Fe and Cu as well as to the average daily intake of phenolics. Furthermore, the higher values for most nutrients measured in the wild species and in the local land races indicate that new pepino varieties with improved fruit contents in nutrient and bioactive compounds can be developed.

Keywords: antioxidants, breeding, diversity, minerals, pigments, *Solanum muricatum*, wild species

1. Introduction

In spite of the potential of underutilized crops to improve human nutrition and to promote the diversification of agriculture, there is very little information available on the diversity of nutritive compounds in underutilized crops and in their wild relatives (National Research Council, 1989; Toledo & Burlingame, 2006). The evaluation of the content of nutrients and bioactive compounds may contribute to the enhancement of neglected crops, given that such studies may result in the discovery of significant or high levels for certain nutrients or bioactive compounds that can enhance market demand. Furthermore, knowledge of the diversity in land races of a cultigen, and of the most closely related wild relatives is of considerable importance for selection and for breeding. As has occurred in many major crops, the evaluation of closely related species can also allow the identification of sources of variation for its utilization in breeding programmes aimed at improving the nutritional and functional quality of neglected crops .

The pepino (*Solanum muricatum* Aiton) is a little known crop from the Andean region cultivated for its fruits and is phylogenetically closely related to tomato and potato (Spooner, Anderson, & Jansen, 1993). Despite being a major crop during pre-Columbian times, as revealed by the Spanish chroniclers and from multiple ancient pottery representations, the New World pepino did not have the impact of other members of the same family (Solanaceae), like tomatoes, potatoes and peppers and was largely substituted by other Old World crops and became a neglected or “lost” crop (National Research Council, 1989; Prohens, Ruiz, & Nuez, 1996). However, during recent decades there has been a renewed interest in pepino cultivation, in particular for diversification of horticultural production, both in its region of origin and in other countries from tropical, subtropical and temperate regions (Rodríguez-Burruezo, Prohens, & Fita, 2011). The pepino fruit, which normally weighs between 150 and 300 g and is typically round, ellipsoid or elongated (Herraiz et al., 2015a), has some attractive characteristics for consumers, like yellow skin covered by purple stripes, intense aroma and yellow juicy flesh with a mild sweet taste (Rodríguez-Burruezo, Prohens, & Fita, 2011). The pepino fruit generally is consumed when fully ripe in the same way as melon, i.e., as a refreshing dessert fruit, although less sweet (Prohens et al., 2005). A less common use is of the less-ripe fruits cut and used in a similar way to cucumber. Infact, its name in Spanish is "pepino dulce", which means "sweet cucumber", while the English name "pepino" was directly taken from the Spanish word for cucumber (Prohens, Ruiz, & Nuez, 1996), presumably because of the similarities of the flesh, scent, and flavor. The pepino recognized for beneficial attributes for human-health, like anti-inflammatory, anticarcinogenic, and antidiabetic properties (Hsu, Guo, Wang, & Yin, 2011; Shathish & Guruvaypoorappan, 2014); obviously, these can contribute to increasing demand.

The composition of pepino has been barely studied and most studies involve only one or very few varieties (Redgwell & Turner, 1986; Fresquet et al., 2001; Prono-Widayat, Schreiner, Huyskens-Keil, & Lüdders, 2003; Prohens et al., 2005; Huyskens-Keil, Prono-Widayat, Lüdders, & Schreiner, 2006; Kola, 2010). These previous studies showed that pepino has a high water content (normally above 90%), a soluble solids content usually between 5% and 8%, a low content of sugars and organic acids (commonly below 4% and 0.5%, respectively), and a significant content of vitamin C (generally between 30 and 80 mg/100 g), generally above those of most tomato varieties (Figàs et al., 2015). Studies involving a larger number of varieties mostly focused on proximate composition traits, like soluble solids content or acidity, or vitamin C content (Rodríguez-Burruezo, Prohens, & Nuez, 2002), revealing a large variation within the cultivated species that could be exploited for selection and breeding (Rodríguez-Burruezo, Prohens, & Fita, 2011).

Wild species have been extensively used for breeding in many crops, including composition traits, where valued characters have been transferred from related wild species or primitive land races via introgressive hybridization to cultigens. The pepino and its closest wild relatives form part of *Solanum* section *Basarthrum*, which includes 15 species (Anderson & Jansen, 1998; Anderson, Martine, Prohens, & Nuez, 2006). Within this section, pepino is the only member of series *Muricata* and hybridizes easily with several species of series *Caripensia* (Rodríguez-Burruezo, Prohens, & Fita, 2011). In particular, hybrids with *S. caripense* Humb. & Bonpl. ex Dun. and *S. tabanoense* Correll are highly fertile and it is possible to obtain backcrosses to pepino (Anderson, 1979; Rodríguez-Burruezo, Prohens, & Fita, 2011). Few works have been done on the composition, other than proximate composition traits, of these wild genetic resources for pepino breeding. The fruits of both species include a higher content in soluble solids, acidity and vitamin C than cultivated pepino (Prohens et al., 2005; Rodríguez-Burruezo, Prohens, & Fita, 2011) and have been used in backcross breeding programmes of pepino aimed at improving the fruit quality (Rodríguez-Burruezo, Prohens, & Fita, 2011). Also, another wild species (*S. trachycarpum* Bitter & Sodiro), may be of special interest for pepino breeding as it grows in dry areas (Anderson, 1975; Anderson, Martine, Prohens, & Nuez, 2006), which may be associated to higher dry matter and concentration of nutrients in the fruit. However, the potential of wild species for pepino breeding for increased content in most nutrients and bioactive compounds is largely unknown and no studies exist on theprotein, phenolics, pigments, and mineral content.

Selection and breeding programmes in pepino have mostly been performed in countries outside of the Andean region, where most of the diversity exists (Blanca et al., 2007; Herraiz et al., 2015a), and have mostly concentrated on yield, taste and adaptation to intensive production systems (Rodríguez-Burruezo, Prohens, & Fita, 2011). As a result of these breeding programmes, several modern varieties have been obtained that are mostly adapted to the new cultivation conditions and environments in which pepino has been introduced (Rodríguez-Burruezo, Prohens, & Fita, 2011; Herraiz et al., 2015a). In a previous study we demonstrated that most modern breeding has resulted in a reduction of the genetic diversity in the modern varieties and also in significant morphological changes compared to local varieties from the Andean region (Herraiz et al., 2015a). In other studies, modern breeding has been linked to a decrease in the concentration of nutrients (Davis, 2009). In the case of pepino, no information exists on the effect of breeding and selection on chemical composition traits that were not the target of the selection programmes. We consider that it is important to evaluate the impact of modern breeding on nutrients and bioactive compounds of pepino, as this may have important implications for the consumers as well as for establishing new breeding objectives (Rodríguez-Burruezo, Prohens, & Fita, 2011).

In this present study, we analyze the content in dry matter, protein, total phenolics, β-carotene, chlorophylls and minerals from a number of accessions of pepino, including local land races and modern varieties (more highly selected for 20th and 21st century marketing), as well as that of several related species. We evaluate the diversity, differences among groups, relationships among accessions and traits, and the potential contribution to the diet resulting from pepino consumption. The study we present will be of interest for in the most general way to show how ‘neglected’ crops have been diminished by modern selection, and specifically how the pepino crops can be enhanced, and made more valuable as a dietary supplement.

2. Material and Methods

2.1. Plant material and cultivation conditions

Twenty-one accessions, corresponding to 15 varieties of pepino and six accessions of three species of wild relatives were used for the present study (Table 1). Pepino materials included seven local land races from the Andean region and eight modern varieties produced through selection and breeding programmes in different countries. Wild relatives consisted in four accessions of the widespread *S. caripense* and one accession of *S. tabanoense* and *S. trachycarpum* (Table 1). A representative sample of fruits of the three groups is displayed in Fig. 1. Previous morphological and molecular characterization of most of these materials revealed that they encompass a wide genetic diversity (Blanca et al., 2007; Herraiz et al., 2015a).

For each accession, five plants were clonally micropropagated and cultivated in order to obtain fruits. Plants were cultivated in a greenhouse with hydroponic facilities (quartz sand benches) in Valencia (Spain) in order to avoid experimental error arising from variation in plant nutrition and differences in the amount of water available to individual plants. Plants were distributed according to a completely randomized design and spaced 1.7 m between rows and 0.4 m apart in the row. A drip irrigation system using pressure compensating emitters were used for providing the nutrient solution, which had the following final concentration of the main anions and cations (resulting from the ions present in the irrigation water plus those added with the soluble fertilizers): 11.47 mM NO_3^-, 1.00 mM NH_4^+, 1.50 mM $H_2PO_4^-$, 6.75 mM K^+, 3.25 mM Ca^{2+}, 2.50 mM Mg^{2+} and 2.82 mM SO_4^{2-}. Microminerals were supplied by adding the following salts to the irrigation water: 50 μM H_3BO_3, 10 μM FeEDTA, 4.5 μM $MnCl_2$, 3.8 μM $ZnSO_4$, 0.3 μM $CuSO_4$ and 0.1 μM $(NH_4)_6Mo_7O_{24}$. In order to stimulate fruit set, flowers were mechanically vibrated, and for self-incompatible wild species *S. caripense* and *S. tabanoense* manual pollinations were performed using pollen from other genotypes of the same species in order to obtain fruits.

2.2. Preparation of samples

Five samples (replications) per accession were taken, with each replication corresponding to fruits of one of the five plants included in the experiment. Fruits were collected when fully ripe. This stage is determined by the fruit having reached the final size and displaying the typical pepino yellow background colour covered by purple/brownish stripes and releasing an intense aroma (Herraiz et al., 2015b). After harvesting, fruits were brought to the laboratory, where they were washed, peeled and cut into longitudinal slices. The fruit slices were weighted and frozen in N_2 and stored at -80ºC until lyophilised. Freeze-dried tissue corresponding to the fruits of each individual plant was bulked and powdered to form each of the samples.

2.3. Analytical methods

Dry matter was determined using the fruit samples weight before and after lyophilisation using the formula 100 x (dry weight/fresh weight). Protein content was calculated as N x 6.25 from the N content values determined with the Kjeldahl method.

Total phenolics (g/100 g) were determined according to the Folin-Ciocalteu procedure (Singleton & Rossi, 1965) after extraction with acetone (70% v/v) and acetic acid (0.5% v/v). Absorbance was measured at 750 nm and caffeic acid (Sigma-Aldrich Chemie) was used as a standard. For β-carotene determination, samples were extracted with ethanol:hexane (4:3 v/v) in darkness. After separation of the hexane phase, β-carotene contents (mg/100 g) were determined by measuring absorbance at 452 nm and 510 nm through the molar extinction coefficient. Chlorophylls *a* (C*a*) and *b* (C*b*) and total chlorophyll were measured spectrometrically after extraction with acetone (80% v/v) according to Wellburn (1994). Basically, the absorbance of the extract was measured at 645 nm (A645) and 663 nm (A663) and the concentrations (mg/100 g) of chlorophylls *a* and *b* were calculated using the following formulas: $Ca=[V\times(12.7\times A663 - 2.69\times A645)]/(10\times W)$ and $Cb=[V\times(22.9\times A645 - 4.68\times A663)]/(10\times W)$, where V is the volume (mL) of the extract and W is the sample weight. Total chlorophylls content was calculated as the sum of C*a* and C*b*.

For the analysis of minerals, 2 g of the liophylised samples were calcined in a furnace at 450ºC for 2 h. Subsequently they were weighted and dissolved in 2 mL of HCl. The mixture was heated until vapors appeared, after which immediately several mL of distilled water were added. After filtration, the extract volume was brought to 100 mL with distilled water. The following methodologies were used for the different minerals: P was determined by spectrometry using the molibdovanadate method, K by flame photometry, and Ca, Mg, Fe, Cu and Zn by atomic absorption spectrophotometry. All results of composition determinations are reported on a 100 g fresh weight basis.

2.4. Data analysis

Data for each composition trait were analyzed using a one-way factorial analysis of variance (ANOVA) and least significant difference (LSD) values were calculated. The average and standard error (SE) were calculated from accession means for pepino land races, pepino modern varieties and wild relatives accessions as well as for the whole collection. Significance of pairwise differences among averages for pepino land races, pepino modern varieties and wild relatives were calculated with *t*-tests. Given that differences among averages of the three groups of accessions for the traits measured could result in overestimated results for the correlations between traits, pairwise correlations were calculated based on within-group residuals of accession means (i.e., intra-group correlation). Principal components analysis (PCA) for all accessions and for pepino varieties only were performed for standardized composition trait using Euclidean distances among accessions.

The contribution (in percentage) of one serving (200 g) of pepino to the daily "Recommended Dietary Allowances" (RDA) for protein, vitamin A, and all minerals except K and the "Adequate Intake" (AI) for K were calculated according to the values for adult males and females of RDA and AI provided by Food and Nutrition Board (2011).

3. Results

3.1. Composition

A great diversity, with highly significant differences ($P<0.0001$) among the set of cultivated and wild accessions studied, was observed for all composition traits studied (Tables 2 and 3). Differences of several-fold, with a minimum of 3.3-fold for dry matter content and a maximum of 111-fold for chlorophyll *a* were observed in the collection. When the comparison is restricted to cultivated pepino, these differences are of a minimum of 1.5-fold for dry matter content and a maximum of 15.5-fold for chlorophyll *a*. The wild species had higher average values for all traits, than the cultivated species. The average values for all characters of the wild species were significantly higher than those of pepino local land races or modern varieties (Tables 2 and 3). In fact, except for β-carotene, chlorophyll *b* and Fe contents, there is not even an overlap between values observed in cultivated and wild species (Tables 2 and 3).

Dry matter content ranged between 5.95 and 8.08 g/100 g in pepino and between 10.50 and 17.28 g/100 g in the wild species, with the highest value corresponding to the single *S. trachycarpum* accession (E-34) (Table 2). No significant differences were observed for average values between local and modern varieties of pepino. For protein content the values for the cultivated pepino varied between 0.365 and 0.652 g/100 g in pepino and 1.247 and 2.027 g/100 g in the wild species (Table 2). Local land races of the pepino varieties possessed significantly higher contents than modern varieties, with the former having an average content 6.3% higher than the latter. In fact, all modern varieties had protein contents below 0.5 g/100 g, while five out of the seven land races presented protein contents above this value (Table 2).

Total phenolics ranged between 50.9 and 123.6 mg/100g in pepino and between 175.4 and 287.6 mg/100 g in the wild species. β-carotene values were much lower with values between 48.8 and 166.1 μg/100 g in pepino and 159.2 and 641.8 μg/100 in the wild species. Chlorophyll *a* content was generally higher than that of chlorophyll *b* in all accessions, with an average ratio of 1.72. The ranges of variation were large in pepino, with total chlorophyll content between 0.112 and 1.234 mg/100 g. This wide range was due to an odd accession (RP-1) with very low content in chloropyll. In this respect, this pepino variety had total chlorophyll content 2.5-fold lower than that of the pepino variety ranking second for lowest chlorophyll values. For wild species, the range of total chlorophyll content ranged from 1.374 to 6.888 mg/100 g. No significant differences were observed between local and modern varieties for any of the antioxidants and pigments evaluated (Table 2).

Among the macrominerals, the highest concentration was found for K , with an average value of 180.6 mg/100 g in the set of accessions (Table 3). P was the second mineral with highest content values, with an average content of 22.01 mg/100 g, followed by Ca and Mg, with average values of 7.01 and 4.98 mg/100 g, respectively. For microminerals, the highest average concentration was for Fe (0.262 mg/100 g), followed by Cu (0.262 mg/100 g) and Zn (0.172 mg/100 g). As occurred for the rest of traits, average values of wild species for all minerals were much higher than those of the cultivated species, with differences ranging from 2.15-fold for Mg to 4.50 for Zn. For all minerals, important differences were observed in the set of accessions and also among pepino or wild accessions (Table 3). For example, for K the range in cultivated pepino was between 49.9 and 176.9 mg/100 g, while in the wild species, the range varied between 212.2 and 432.1 mg/100 g. For Cu, the relative variation was very large in the cultivated pepino with contents varying from 0.004 to 0.047 mg/100 g, although in absolute values it was larger in the wild species, in which it ranged between 0.053 and 0.131 mg/100 g. Although the ranges of variation between local and modern pepino varieties overlapped for all minerals, on average, the land races possessed significantly higher contents in P (33.9%), K (52.3%) and Zn (61.2%) than modern varieties (Table 3).

3.2. Correlations among traits

A total of 41 out of 91 pairwise correlations, calculated from the within-group residuals of accessions means, were significant (Table 4). Dry matter and protein were positively correlated and both of exhibited positive correlations with β-carotene, as well as with minerals P, Mg and Zn. Dry matter was also positively correlated with total phenolics, while protein content with K. Total phenolics were positively correlated with the minerals Ca, Fe and Zn (Table 4). The pigments β-carotene, chlorophylls *a* and *b*, and total chlorophylls were positively intercorrelated, and all of them were also correlated with K and Mg contents. β-carotene was positively correlated with P, while the chlorophylls were negatively correlated with Cu. Regarding correlations among minerals, P was positively correlated with Mg and Zn, K was positively correlated with Mg and negatively with Fe and Cu, and Mg presented a positive correlation with Zn. Finally, the three microminerals (Fe, Cu and Zn) were positively intercorrelated.

3.3. Principal components analysis

The first component of the PCA with all accessions accounted for 84.0% of the variation and had an eigenvalue of 11.76, while the second component barely accounted for 9.2% of the variation, with an eigenvalue of 1.28 (see Supplementary material). All the composition traits were positively correlated with the first component, with values between 0.230 (for Cu) and 0.286 (for Protein). The second PCA component was highly positive (>0.2) correlations with β-carotene, the three chlorophyll measures, and K, and high negative (<-0.2) correlations with Ca, and especially Fe and Cu (see Supplementary material). The PCA plot with all accessions shows that the first component clearly separates the wild species, with highly positive values for the first component, from the pepino accessions that had negative values for this first component (Fig. 2). The second component does not separate the different groups, although most pepino and wild accessions had positive values. The PCA plot with all accessions also reveals that wild species have a greater dispersion than pepino in both the first and second components (Fig. 2).

The first and second components of an additional PCA, which included only pepino varieties, accounted for 37.4% and 24.6% of the total variation, respectively (see Supplementary material). Eigenvalues for the first and second components were 5.23 and 3.44 respectively. The first component was positively correlated with all traits, except with Ca, Fe and Cu, which had small absolute values (<0.08 in all cases). The second component had very high positive correlations with Ca (0.508), Fe (0.514) and Cu (0.498), while it had a highly negative correlation with K (-0.302) (see Supplementary material). The PCA graph revealed that land races of pepino presented a greater dispersion than modern varieties in both the first and second components (Fig. 2). All modern varieties presented negative values for the first component, while land races presented a wide range of values, with one variety showing highly positive values (OV-8), three varieties intermediate positive values (CH2-22, Col-1 and OT-1), two with values close to 0 (37-A and PT-154) and one with highly negative values (RP-1) (Fig. 2). When considering the second component, the land races were separated in three groups, with 37-A having highly positive values, PT-154 and RP-1 moderate negative values and values close to 0 for the rest of varieties . The modern varieties were separated in two clusters, one with positive values for the second component (El Camino, Puzol and Valencia) and another with negative values, which included the rest of varieties (Fig. 2).

3.4. Contribution to RDA/IA

The comparison of the nutrient values contained in one serving (200 g) of pepino and the nutrients included in the RDAs and AIs by the Food and Nutrition Board (2011) are presented in Table 5. The results reveal that on average one serving of pepino makes a low contribution (<3%) to the protein, vitamin A, Ca, Mg, and Zn RDAs for both male and female adults and to the Fe RDA for females (Table 6). However, for P, K and Cu for adults of both sexes and Fe for adult males, one serving of pepino provides a moderate contribution (3-6%) to the RDA (and AI for K). When considering the best pepino variety for each of the nutrients, the contribution is low (<3%) for protein, Ca and Mg for both sexes and Zn for males, moderate (3-6%) for vitamin A, Fe and Zn for adult females, and considerable (>6%) for P, K and Cu for both sexes and for Fe for adult males (Table 5).

4. Discussion

Selection and breeding for pepino varieties with improved content in nutrients and bioactive compounds is an important objective for the enhancement of this neglected crop (Rodríguez-Burruezo, Prohens, & Fita, 2011). This is the first work in which a large diversity of cultivated pepino and some of its closest wild relatives has been examined for traits such as protein content, β-carotene, total phenolics, and content in minerals. Therefore, it represents an important contribution to the identification of sources of variation for selection and breeding programmes.

The ranges of values obtained by us are in agreement with previous studies in which the composition of one or a few varieties of pepino has been studied (Redgwell &

Turner, 1986; Fresquet et al., 2001; Prono-Widayat, Schreiner, Huyskens-Keil, & Lüdders, 2003; Prohens et al., 2005; Huyskens-Keil, Prono-Widayat, Lüdders, & Schreiner, 2006; Kola, 2010), confirming that pepino has a high water content and a low protein content. Compared to other fleshy fruits with uses similar to those of pepino, like melon (*Cucumis melo* L.; as a fresh fruit) or cucumber (*Cucumis sativus* L.; for using salads) (Prohens, Ruiz, & Nuez, 1996), it has similar contents of dry matter, protein, and minerals (Ekholm et al., 2007; Maynard & Hochmuth, 2007; Maietti et al., 2012). However, the content of total phenolics is much higher than that of both melon and cucumber (Fu et al., 2011; Maietti et al., 2012). Compared to other solanaceous berries, it presents a somewhat higher content of phenolics than tomato (Figàs et al., 2015) or eggplant (Raigón, Prohens, Muñoz-Falcón, & Nuez, 2008), suggesting that it has a high antioxidant capacity. The content of β-carotene it is also similar to that of honeydew melons (*C. melo* var. *inodorus*), although much lower than that of the cantaloupe type (*C. melo* var. *cantalupensis*) (Laur & Tian, 2011), and higher than that of standard commercial types of cucumber (Cuevas, Song, Staub, & Simon, 2010). Regarding the content in chlorophylls, it is similar to that of non-green-fleshed melons (Reid, Lee, Pratt, & Chichester, 1970) and lower than that of cucumber (Chen & Yang, 2012). These comparisons suggest that for the traits we have evaluated, the pepino presents an overall composition similar to that of melon and cucumber, although with a much higher content in phenolics. This specific difference in concentration of phenolics may be of relevance for the promotion and enhancement of pepino, as there is an increasing demand for fruit and vegetable crops with higher bioactive compound content (Diamanti, Battino, & Mezzetti, 2011).

The results reveal that there is a great compositional diversity in pepino fruits , matching the results obtained for morphological traits and molecular markers (Blanca et al., 2007; Herraiz et al., 2015a), indicating that there are ample opportunities for selection and breeding. Considerable differences were found between the composition of cultivated pepino and its wild relatives. Compared to the cultivated pepino, wild species bear a higher dry matter content, as well as higher concentrations for the rest of traits studied, than the cultivated pepino. Other studies have found that wild relatives *S. caripense* and *S. tabanoense* bear higher concentrations of dry matter than the cultivated species (Prohens et al., 2005). Amazingly, although the dry matter content of wild species was higher than that of the cultivated pepino, it only accounted partially for the larger values observed in the rest of nutrients for the wild species. That is, the ratio between the average content in dry matter between wild relatives and the cultivated pepino was lower (in some cases much lower) than that for the rest of traits. For example, while on average the dry matter content was less than two-fold higher in the wild species compared to the cultivated pepino, the β-carotene, chlorophylls, and Zn were greater than four-fold higher. This suggests that the pepino wild relatives that we have evaluated, that are cross-compatible with pepino (Anderson, 1975, 1979; Rodríguez-Burruezo, Prohens, & Fita, 2011), may represent a very useful source of variation for pepino breeding. In particular, *S. trachycarpum* fruits have a dry matter content almost three-fold higher than that of pepino. This species is from dry areas (Anderson, 1975, Anderson, Martine, Prohens, & Nuez, 2006) and probably has acquired the capacity to accumulate higher contents of nutrients in the fruit than other species, even when grown under the same conditions in which the supply of water is not a limiting factor. This makes *S. trachycarpum* an interesting genetic resource for pepino breeding, not only because it can help increasing the pepino fruit quality, but also for adaptation to drought.

Within the cultivated pepino there have been also many differences among varieties for the composition traits. Large variations for composition traits within the cultivated species have also been observed for other crops of the same family, like tomato or eggplant (Raigón, Prohens, Muñoz-Falcón, & Nuez, 2008; Figàs et al., 2015). In pepino, these differences have always been greater for composition traits than for dry matter content, indicating that considerable genetic differences exist in the capacity to accumulate certain nutrients or bioactive compounds and therefore clonal selection can be successfully applied (Rodríguez-Burruezo, Prohens, & Fita, 2011). This suggests that some pepino clones, like OV-8, which ranks first for total phenolics, β-carotene, chlorophylls, P and Zn and presents high or intermediate values for the rest of traits, would be a good candidate for selection of a clone with high content in nutrients and bioactive compounds. In some cases, such as chlorophyll *a* and total chlorophylls and Cu, the differences have been of more than 10-fold within the cultivated species. For all traits a continuous range of variation has been found, but in the case of chlorophyll content one variety (RP-1), the contents of both chlorophyll *a* and *b* are very low, suggesting that it may be a mutant for deficit of chlorophyll content in the fruit flesh. In other crops, like cucumber or melon, mutants for low chlorophyll content have been described (Cuevas, Song, Staub, & Simon, 2010; Dogimont, 2011), and in the case of pepino this could be of interest for selecting varieties with flesh with higher yellow chroma and luminosity.

There is evidence that modern breeding has resulted in a reduced concentration of nutrients in modern varieties of fruits and vegetables as a result of the so-called "dilution effect" attributable to the higher yields of modern varieties (Davis, 2009). With the pepino, we have found a significantly lower content in protein, P, K and Zn in the modern pepino varieties compared to the land races, which may be a consequence of the selection for high yield of the modern pepino varieties (Rodríguez-Burruezo, Prohens, & Fita, 2011). In a previous study, we also found that modern breeding resulted in the selection of clones with notably different morphological differences vs. land races (Herraiz et al., 2015a), likely resulting from selection for adaptation to new environments (Rodríguez-Burruezo, Prohens, & Fita, 2011). The multivariate analyses we have performed also confirm that, as reported for morphological traits and molecular data (Herraiz et al., 2015a), modern pepino varieties possess a lower compositional diversity vs. land races. As a consequence, as has been done in other crops, we suggest that breeding programmes should also take into consideration the nutrient composition in addition to yield and organoleptic quality (Diamanti, Battino, & Mezzetti, 2011)

Results obtained for within-group correlations are of interest, as they may result from pleiotropy, and, as a result traits under direct selection may result in indirect selection of other correlated traits as well. For the pepino, most of the significant correlations observed have been positive, which may be advantageous for selecting increased concentration of nutrients and bioactive compounds. For example, selection for higher content of phenolics, an increasingly important breeding objective in fruits and vegetables (Kaushik et al., 2015), may result in indirect selection for increased β-carotene, P, Mg and Zn contents, that are desirable in order to improve the nutrient composition of pepino fruits. Also, we recorded an expected positive correlation between chlorophylls and Mg, as chlorophyll molecules contain an Mg ion. The most important negative correlations observed between Cu and chlorophyll content, is not a surprise given that Cu is known to induce chlorophyll loss (Ouzounidou, 1996).

Discovering or highlighting composition properties is of great relevance for the enhancement of new crops, as consumers increasingly value knowledge of such constitutents. The comparison of the composition values obtained with the RDA/AI

(Food and Nutrient Board, 2011) for the different nutrients studied shows that the pepino may noticeably contribute to P, K, Fe and Cu intakes; this information could obviously be exploited for promoting this crop. In particular, one serving of selected pepino varieties could provide more than 10% of the RDA of Cu for both sexes or of Fe for males. For total phenolics, there is no RDA/AI; however, the consumption in different European populations has been estimated at around 800-900 mg/day on average (Tresserra-Rimbau et al., 2013). On average, one serving of 200 g of pepino represents around 20% of the daily consumption of total phenolics; clearly that is a considerable contribution. Furthermore, if the varieties with higher content are used in breeding and selection programs, this percentage could increase to almost 30%. Given the benefits of dietary phenolics to human health, the high content in phenolics of the pepino also may make it an attractive fruit for health-concerned consumers. The high content in phenolics may also be related to the healthy properties attributed to pepino (Hsu, Guo, Wang, & Yin, 2011; Shathish & Guruvaypoorappan, 2014).

5. Conclusions

Our results provide information on the composition and diversity of pepino fruit for dry matter, total phenolics, β-carotene, chlorophylls and minerals. Overall the results showed that the pepino is a highly diverse crop for fruit composition, indicating that there is a high potential for selection and breeding. Also, wild related species represent interesting sources of variation for pepino breeding, as they bear much higher values than those present in the cultivated species. The fact that modern varieties of pepino express less diversity for fruit composition and contain lower amounts of protein, P, K, and Zn, than local land races suggests that modern breeding programmes should include these species as well in the effort to produce fuits with higher values of nutrients and bioactive compounds. Finally, the high content in phenolics of the pepino may be exploited for its promotion as a valuable entry in the health-conscious natural fruit and vegetable commodity market.

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Table 1

Plant materials, origin and fruit characteristics of the pepino materials and wild relatives used.

Accession	Code	Species	Country of origin/breeding	Fruit length (cm)	Fruit width (cm)	Fruit length/width ratio
Local land races of the pepino						
37-A	37	*S. muricatum*	Ecuador	7.3±0.2	4.1±0.1	1.79±0.05
Col-1	Co	*S. muricatum*	Colombia	7.3±0.2	7.9±0.2	0.93±0.03
CH2-22	CH	*S. muricatum*	Chile	7.8±0.9	7.7±1.0	1.03±0.04
OT-1	OT	*S. muricatum*	Ecuador	6.0±0.3	5.5±0.3	1.09±0.02
OV-8	OV	*S. muricatum*	Chile	5.7±0.5	5.6±0.1	1.03±0.09
PT-154	PT	*S. muricatum*	Peru	7.9±0.6	11.1±1.2	0.72±0.03
RP-1	RP	*S. muricatum*	Ecuador	5.6±0.4	8.1±0.4	0.71±0.07
Modern pepino varieties						
El Camino	EC	*S. muricatum*	New Zealand	7.6±0.7	6.0±0.7	1.27±0.04
Kawi	Ka	*S. muricatum*	New Zealand	14.5±0.9	9.4±0.6	1.55±0.03
Puzol	Pu	*S. muricatum*	Spain	10.9±0.4	6.8±0.3	1.60±0.08

Quito	Qu	*S. muricatum*	United Kingdom	6.6±0.4	6.1±0.4	1.08±0.01
Sweet Long	SL	*S. muricatum*	Spain	10.8±0.5	5.7±0.3	1.89±0.08
Sweet Round	SR	*S. muricatum*	Spain	7.2±0.4	8.3±0.3	0.87±0.05
Turia	Tu	*S. muricatum*	Spain	15.5±0.7	7.4±0.1	2.08±0.08
Valencia	Va	*S. muricatum*	Spain	11.6±1.2	5.3±0.6	2.21±0.20
Wild relatives						
BIRM/S 1034	c1	*S. caripense*	Ecuador	2.8±0.1	2.7±0.1	1.01±0.01
E-7	c2	*S. caripense*	Ecuador	3.5±0.1	3.6±0.1	0.97±0.02
EC-40	c3	*S. caripense*	Ecuador	2.8±0.2	2.8±0.1	1.01±0.03
QL-013	c4	*S. caripense*	Ecuador	3.2±0.1	2.9±0.1	1.10±0.02
E-257	ta	*S. tabanoense*	Ecuador	4.6±0.1	3.7±0.1	1.26±0.03
E-34	tr	*S. trachycarpum*	Ecuador	2.5±0.1	2.1±0.1	1.18±0.03

Values are expressed as mean ± S.E. of five independent samples for each variety.

Table 2

Mean values for dry matter, protein content, total phenolics, β-carotene, and chlorophylls on a fresh weight basis of 15 accessions of local land races and of modern pepino varieties and six accessions of wild relatives. Statistics included are: average ±SE for the three groups of accessions (local, modern, wild) and for the global mean, and values of the *F*-test for differences among accessions and least significant differences (LSD, P=0.05).

Accession	Dry matter (g/100 g)	Protein (g/100 g)	Total phenolics (mg/100 g)	β-carotene (μg/100 g)	Chlorophyll *a* (mg/100 g)	Chlorophyll *b* (mg/100 g)	Total chlorophyll (mg/100 g)
Local land races of the pepino							
37-A	5.95	0.565	107.2	82.6	0.448	0.218	0.665
Col-1	7.61	0.589	78.6	93.3	0.469	0.297	0.765
CH2-22	8.08	0.565	73.1	133.0	0.332	0.143	0.474
OT-1	7.00	0.652	103.3	107.3	0.385	0.256	0.641
OV-8	7.17	0.649	123.6	166.1	0.619	0.616	1.234
PT-154	6.22	0.448	87.5	113.3	0.226	0.221	0.447

RP-1	6.03	0.399	70.3	55.9	0.040	0.072	0.112
Average	6.87±0.31	0.552±0.036	91.9±7.5	107.4±13.5	0.360±0.070	0.2603±0.066	0.620±0.130
Modern pepino varieties							
El Camino	7.30	0.497	96.7	91.2	0.338	0.283	0.620
Kawi	5.26	0.401	49.4	40.0	0.429	0.385	0.813
Puzol	6.91	0.386	82.1	109.7	0.402	0.269	0.670
Quito	6.14	0.411	72.4	75.3	0.394	0.515	0.909
Sweet Long	6.35	0.365	114.2	90.6	0.318	0.224	0.542
Sweet Round	7.30	0.489	84.9	118.9	0.470	0.393	0.863
Turia	5.93	0.423	50.9	103.2	0.481	0.359	0.840
Valencia	6.51	0.399	89.9	48.8	0.180	0.105	0.285
Average	6.46±0.25	0.421±0.017	80.1±7.8	84.7±9.99	0.377±0.035	0.317±0.044	0.693±0.074
Wild relatives							

BIRM/S 1034	12.09	1.511	199.3	399.0	3.087	1.588	4.674
E-7	12.45	1.379	287.6	159.2	0.865	0.510	1.374
EC-40	10.50	1.247	215.9	483.6	4.193	2.696	6.888
QL-013	12.63	1.786	205.3	554.7	2.852	0.913	3.765
E-257	11.12	1.607	175.4	479.0	3.422	1.087	4.508
E-34	17.28	2.027	284.5	641.8	4.447	3.070	7.515
Average	12.68±0.98	1.593±0.115	228.0±19.2	452.9±67.6	3.144±0.522	1.644±0.419	4.787±0.906
Global mean	8.37	0.800	126.3	197.4	1.162	0.677	1.838
Prob. *F*-test	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
LSD (P=0.05)	1.13	0.190	29.6	92.5	0.408	0.118	0.690
Prob. values for *t*-test for averages comparison							
Local vs. modern	0.3239	0.0049	0.2975	0.1926	0.8270	0.4775	0.6216
Local vs. wild	<0.0001	<0.0001	<0.0001	0.0002	0.0001	0.0047	0.0004

Modern vs. wild	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0032	0.0002

Table 3

Mean values for mineral content on a fresh weight basis of 15 accessions of local land races and of modern pepino varieties and six accessions of wild relatives. Statistics included are: average ±SE for the three groups of accessions (local, modern, wild) and for the global mean, as well as values of the *F*-test for differences among accessions and least significant differences (LSD, P=0.05).

Accession	P (mg/100 g)	K (mg/100 g)	Ca (mg/100 g)	Mg (mg/100 g)	Fe (mg/100 g)	Cu (mg/100 g)	Zn (mg/100 g)
Local land races of the pepino							
37-A	13.84	96.1	8.94	3.26	0.431	0.047	0.117
Col-1	14.06	167.2	4.26	4.65	0.173	0.023	0.118
CH2-22	15.52	176.9	4.27	4.40	0.182	0.027	0.123
OT-1	20.25	166.7	4.85	4.71	0.133	0.021	0.114
OV-8	25.54	164.6	3.75	3.79	0.188	0.016	0.142
PT-154	14.93	138.1	3.40	3.71	0.157	0.007	0.083
RP-1	10.66	127.7	2.74	3.60	0.124	0.004	0.057
Average	16.40±1.87	148.2±10.9	4.60±0.77	4.02±0.21	0.198±0.040	0.021±0.005	0.108±0.011

Modern pepino varieties							
El Camino	15.38	67.6	8.62	3.78	0.301	0.036	0.078
Kawi	13.04	131.4	5.04	3.65	0.143	0.022	0.081
Puzol	12.34	49.9	8.12	3.49	0.292	0.033	0.075
Quito	12.19	119.1	3.20	3.46	0.119	0.008	0.072
Sweet Long	9.79	115.1	5.04	3.19	0.123	0.007	0.059
Sweet Round	9.00	115.1	3.05	2.83	0.101	0.011	0.050
Turia	12.34	119.3	4.46	4.11	0.112	0.018	0.061
Valencia	13.88	60.9	7.37	3.52	0.249	0.043	0.063
Average	12.25±0.73	97.3±11.4	5.61±0.77	3.50±0.14	0.180±0.030	0.022±0.005	0.067±0.004
Wild relatives							
BIRM/S 1034	41.20	320.8	9.94	6.90	0.365	0.084	0.387
E-7	36.58	212.2	13.53	6.76	0.587	0.123	0.411
EC-40	36.01	354.7	12.91	7.86	0.303	0.059	0.229

QL-013	46.55	336.2	9.24	7.41	0.531	0.131	0.475
E-257	40.84	320.9	9.39	7.76	0.371	0.054	0.296
E-34	48.17	432.1	15.13	11.70	0.528	0.065	0.532
Average	41.56±2.04	329.5±28.9	11.69±1.02	8.07±0.75	0.447±0.047	0.086±0.014	0.388±0.046
Global mean	22.01	180.6	7.01	4.98	0.262	0.040	0.172
Prob. *F*-test	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
LSD (P=0.05)	4.58	34.8	1.85	0.91	0.095	0.027	0.041
Prob. values for *t*-test for averages comparison							
Local vs. modern	0.0480	0.0069	0.3701	0.0567	0.7155	0.8240	0.0025
Local vs. wild	<0.0001	<0.0001	0.0001	0.0002	0.0019	0.0006	<0.0001
Modern vs. wild	<0.0001	<0.0001	0.0004	<0.0001	0.0003	0.0004	<0.0001

Table 4

Pairwise Pearson linear correlations based on within-group residuals of accession means (n=21) for the composition traits studied.

	Protein	Total phenolics	β-carotene	Chlorophyll a	Chloropyll b	Total chlorophyll	P	K	Ca	Mg	Fe	Cu	Zn
Dry matter	0.781^{***}	0.549^{***}	0.448^{*}	0.239^{ns}	0.389^{ns}	0.318^{ns}	0.503^{*}	0.425^{ns}	0.330^{ns}	0.749^{***}	0.323^{ns}	0.051^{ns}	0.772^{***}
Protein		0.233^{ns}	0.658^{**}	0.376^{ns}	0.267^{ns}	0.340^{ns}	0.767^{***}	0.532^{*}	0.108^{ns}	0.672^{***}	0.305^{ns}	0.086^{ns}	0.797^{***}
Total phenolics			-0.120^{ns}	-0.196^{ns}	0.176^{ns}	-0.030^{ns}	0.219^{ns}	-0.137^{ns}	0.594^{**}	0.255^{ns}	0.506^{*}	0.238^{ns}	0.471^{*}
β-carotene				0.858^{***}	0.636^{***}	0.788^{***}	0.558^{**}	0.782^{***}	-0.052^{ns}	0.614^{**}	-0.139^{ns}	-0.362^{ns}	0.252^{ns}
Chlorophyll a					0.852^{***}	0.970^{***}	0.318^{ns}	0.793^{***}	0.067^{ns}	0.605^{***}	-0.315^{ns}	-0.606^{**}	-0.070^{ns}
Chlorophyll b						0.954^{***}	0.246^{ns}	0.733^{***}	0.266^{ns}	0.684^{***}	-0.246^{ns}	-0.574^{**}	0.035^{ns}
Total chlorophyll							0.297^{ns}	0.796^{***}	0.162^{ns}	0.665^{***}	-0.295^{ns}	-0.615^{**}	-0.024^{ns}
P								0.403^{ns}	0.087^{ns}	0.474^{*}	0.219^{ns}	0.146^{ns}	0.647^{**}
K									-0.242^{ns}	0.704^{***}	-0.486^{*}	-0.567^{**}	0.214^{ns}
Ca										0.336^{ns}	0.723^{***}	0.330^{ns}	0.226^{ns}
Mg											0.034^{ns}	-0.311^{ns}	0.468^{*}
Fe												0.749^{***}	0.547^{*}

Cu	0.463*

ns, *, **, *** indicate non-significant, or significant at P=0.05, 0.01 and 0.001, respectively.

Table 5

Contribution from pepinos to a) the daily Recommended dietary allowances (RDAs) for protein, vitamin A and all minerals studied except K, and b) Adequate intake (AI) for K (Food and Nutrition Board, 2011) from a serving size (200 g) of fruit considering the average for all accessions as well as the varieties with highest value for each nutrient. For vitamin A we have considered that it is synthesized exclusively from β-carotene. Data are based only on cultivated (*S. muricatum*) accessions.

			Contribution of one serving (200 g) to daily RDA/AI (%)			
	Daily RDA/AI		Pepino average		Pepino variety with highest value	
Nutrient	Males[a]	Females[a]	Males	Females	Males	Females
Protein (g)	56	46	1.7	2.1	2.3	2.8
Vitamin A[b] (μg)	10800	8400	1.8	2.3	3.1	4.0
P (mg)	700	700	4.1	4.1	7.3	7.3
K (mg)	4700	4700	5.2	5.2	7.5	7.5
Ca (mg)	1000	1000	1.0	1.0	1.8	1.8
Mg (mg)	420	320	1.8	2.3	2.2	2.9
Fe (mg)	8	18	4.7	2.1	10.1	4.8

Cu (mg)	0.9	0.9	4.8	4.8	10.5	10.5
Zn (mg)	11	8	1.6	2.2	2.6	3.5

[a]Values corresponding to adult males and females in the range of 19-50 y. For Mg the value indicated corresponds to the range 31-50 y, which is slightly higher than that for adults of 19-50 y.

[b]Expressed as β-carotene equivalents.

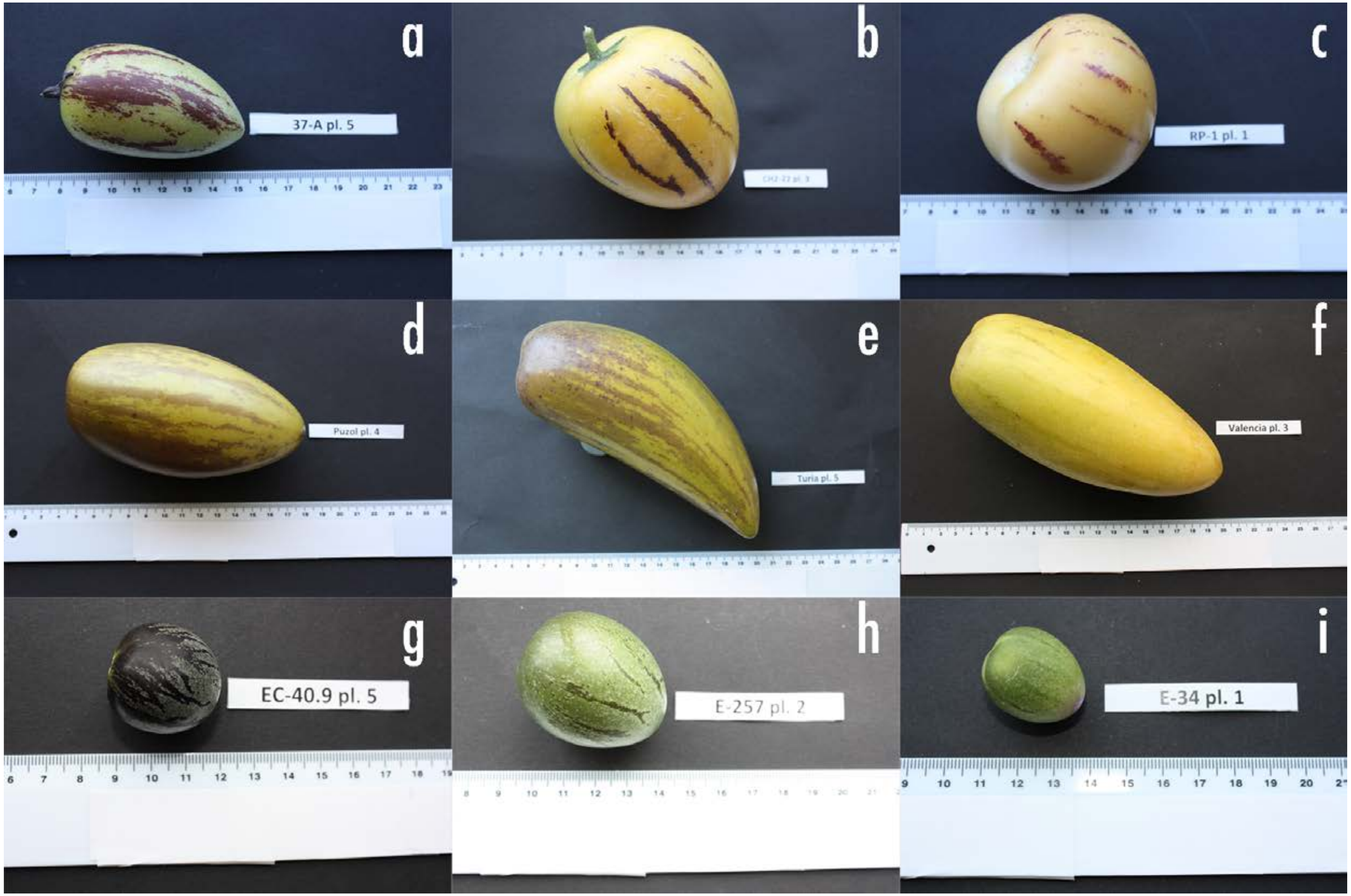
Figure captions

Fig. 1

Fruits of pepino and wild relatives: i) pepino local land races (above) accessions 37-A (a), CH2-22 (b), and RP-1 (c); ii) pepino modern varieties (middle) accessions Puzol (d), Turia (e) and Valencia (f); iii) wild species (below) accessions EC-40 (*S. caripense*; g), E-257 (*S. tabanoense*, h) and E-34 (*S. trachycarpum*; i).

Fig. 2

Principal components analysis scatterplot against the first (X-axis) and second (Y-axis). The principal components are based on 14 fruit composition traits of (a) 15 pepino varieties and six accessions of wild relatives (first and second components account, respectively, for 84.0% and 9.2% of the total variation), and (b) 15 pepino varieties only (first and second components account, respectively, for 37.4% and 24.6% of the total variation). The different groups of accessions are represented by different symbols: pepino local land races (solid circle), pepino modern accessions (open square), and wild *S. caripense* (grey circle), *S. tabanoense* (grey square) and *S. trachycarpum* (grey triangle). See Table 1 for the codes of individual accessions.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37-A pl. 5
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RP-1 pl. 1
d
Puzol pl. 4
e
Turia pl. 5
f
Valencia pl. 3
g
EC-40.9 pl. 5
h
E-257 pl. 2
i
E-34 pl. 1

Fig. 1

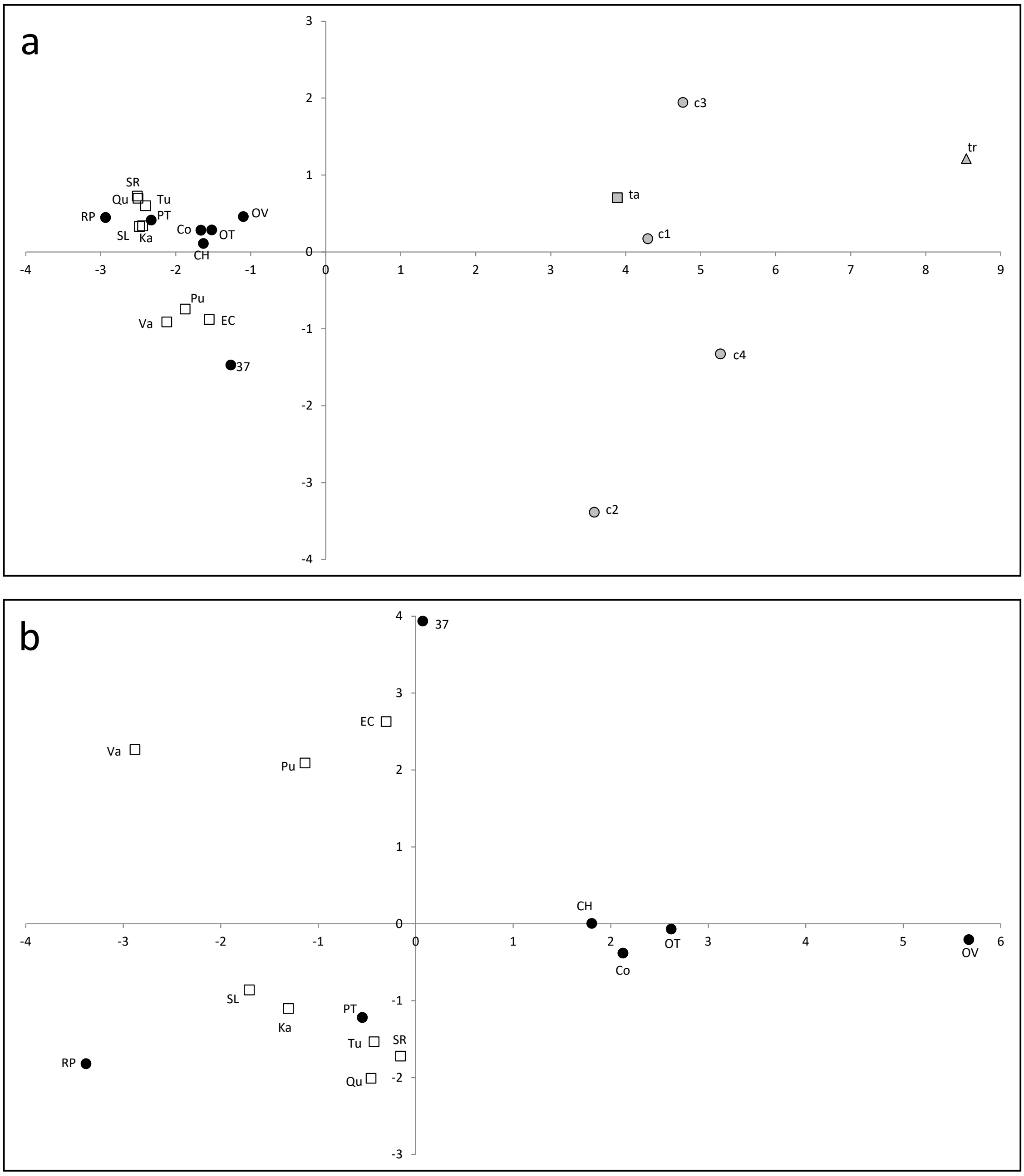
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Fig. 2