# Morphological and Physico-Chemical Characteristics of Fruits of Pepino (*Solanum muricatum*), Wild Relatives (*S. caripense* and *S. tabanoense*) and Interspecific Hybrids. Implications in Pepino Breeding

J. Prohens<sup>1</sup>), M.C. Sánchez<sup>2</sup>), A. Rodríguez-Burruezo<sup>1</sup>), M. Cámara<sup>2</sup>), E. Torija<sup>2</sup>) and F. Nuez<sup>1</sup>)

(<sup>1)</sup>Centro de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV), Universidad Politécnica de Valencia, Valencia, Spain and <sup>2)</sup>Departamento de Nutrición y Bromatología II, Facultad de Farmacia, Universidad Complutense de Madrid, Madrid, Spain)

# Summary

Wild species could be useful for the genetic improvement of pepino (Solanum muricatum Aiton) fruit quality. We studied several agronomic and physico-chemical traits in accessions of S. muricatum (EC-37), wild relatives Solanum caripense (EC-40) and Solanum tabanoense (EC-26), and their corresponding interspecific hybrids. Interspecific hybrids involving EC-37 had yield and fruit weight intermediate between the cultivated and the wild parents, while yield and mean fruit weight of EC-37×EC-26 were greater than those of EC-40×EC-37. Dry matter content (DM) was higher in the wild accessions (>13 %) than in the cultivated pepino (<10 %). Wild species had similar (S. caripense) or lower (S. tabanoense) concentrations of total sugars than S. muricatum. EC-37 and EC-26×EC-37 had similar relative concentrations of the soluble sugars glucose, fructose, and sucrose, while sucrose was predominant (>60 %) in the rest of the tested species and hybrids. Concentration of organic acids was much higher in EC-26 and EC-40 (total acids  $\geq 2500 \text{ mg} 100 \text{ g}^{-1}$  and pH<4.0) than in EC-37 (275 mg 100 g<sup>-1</sup> and pH=5.36), while interspecific hybrids had concentrations more similar to S. muricatum than to wild species. Citric acid was the predominant organic acid in the composition of all the studied accessions (>75 % of total acids). Ascorbic acid content of S. caripense and some interspecific hybrids was more than three times greater than that of S. muricatum. The results indicate that the studied wild species can be utilized to modify and improve the composition of pepino, especially by increasing acidity and ascorbic acid content.

#### Zusammenfassung

Morphologische und physikalisch-chemische Merkmale von Früchten von Pepino (Solanum muricatum), verwandten Wildarten (S. caripense und S. tabanoense) und ihren interspezifischen Hybriden. Auswirkungen auf die Pepino-Züchtung. Wildarten könnten für die Verbesserung des genetischen Hintergrunds der Fruchtqualität von Pepinos (Solanum muricatum Aiton) nützlich sein. Wir haben zahlreiche anbautechnische und physikalisch-chemische Eigenschaften in Akzessionen von S. muricatum (EC-37) und den verwandten Wildarten, Solanum caripense (EC-40) und Solanum tabanoense (EC-26), sowie den jeweiligen interspezifischen Hybriden untersucht. Die Erntemengen und durchschnittlichen Fruchtgewichte lagen bei interspezifischen Hybriden mit EC-37 zwischen denen der Kultur- und Wildart, wobei die von EC-37×EC-26 größer waren als jene von EC-40×EC-37. Die Trockenmasse (DM) war in Wildvorkommen (>13 %) höher als in Kulturpepinos (<10 %). Die Wildarten wiesen gleiche (*S. caripense*) oder geringere (*S. tabanoense*) Gesamt-zucker-Konzentrationen auf als *S. muricatum*. EC-37 und EC-26×EC-37 hatten ähnliche relative Konzentrationen der löslichen Zucker Glukose, Fruktose und Saccharose, wohingegen die Saccharose in den restlichen Arten und Hybriden vorherrschend (>60 %) war. Die Konzentrationen organischer Säuren waren in EC-26 und EC-40 (Gesamtsäuren  $\geq 2500 \text{ mg } 100 \text{ g}^{-1}$  und pH<4.0) höher als in EC-37 (275 mg 100 g<sup>-1</sup> und pH=5.36), die Konzentrationen in den interspezifischen Hybriden lagen näher bei S. muricatum als bei den Wildarten. Zitronensäure war in allen Vorkommen die vorherrschende Säure (>75 % der Gesamtsäure). Der Ascorbinsäure-Gehalt von S. caripense und einiger interspezifischen Hybriden war drei Mal höher als in S. muricatum. Die Ergebnisse weisen darauf hin, dass die untersuchten Wildarten zur Veränderung und Verbesserung der Zusammensetzung von Pepinos, vor allem im Hinblick auf einen höheren Gesamtsäure- und Ascorbinsäuregehalt, geeignet sind.

Key words. flavour - hybridisation - inheritance - organic acids - sugars - ascorbic acid.

# Introduction

The improvement of flavour and nutritional value of pepino fruit (*Solanum muricatum* Aiton) is essential for its successful introduction as a new crop in Europe and North America (WELLES 1992). As in other *Solanaceae* vegetable crops (KALLOO and BERGH 1993), wild related species may be useful for the improvement of pepino fruit quality. *Solanum caripense* Humb. et Bonpl. ex Dunal and *Solanum tabanoense* Correll are wild relatives of pepino (ANDER-SON et al. 1996) and give fertile hybrids when crossed with *S. muricatum* (PROHENS et al. 2003). Fruits from both wild species are harvested and eaten in some rural areas of the Andean region, the centre of origin of pepino and their wild relatives (ANDERSON et al. 1996), because of their intense flavour (HEISER 1985; NUEZ et al. 1999).

The study of fruit quality traits in *S. caripense, S. ta*banoense and interspecific hybrids may provide relevant information on their value as sources of variation for the improvement of fruit quality in the cultivated species. In this sense, as in other fruits, the content in sugars and acids, as well as the ratio between total sugars and total acids, are determinant traits for pepino fruit flavour (STEVENS et al. 1977; EL-ZEFTAWI et al. 1988). Additionally, sugars and acids differ in their sweetness and acidity (KILCAST et al. 2000; O'NEIL et al. 2001).

Several studies carried out on different varieties revealed that ripe pepino fruits contain similar concentrations of glucose, fructose, and sucrose (REDGWELL and TURNER 1986; SCHAFFER et al. 1989; HUYSKENS-KEIL et al. 2000; SÁNCHEZ et al. 2000; PRONO-WIDAYAT et al. 2003). Nevertheless, the composition of organic acids in fruit has been less comprehensively studied, although the findings of REDGWELL and TURNER (1986) and HUYSKENS-KEIL et al. (2000) reveal that citric acid is the predominant organic acid in pepino fruit.

Regarding *S. caripense* and *S. tabanoense*, there is no information about their sugars and acids content. In the case of tomato, a species phylogenetically close to pepino (LESTER 1991), quantitative and qualitative differences have been reported regarding the concentrations of sugars and acids between the cultivated and several wild related species (ROSELLÓ et al. 2002). These differences may be used for the improvement of fruit taste.

Nutritional quality and, in particular, the content in antioxidants is another key trait in the improvement of pepino fruit quality. Pepino fruit has high levels of ascorbic acid (REDG-WELL and TURNER 1986; RODRÍGUEZ-BURRUEZO et al. 2002). However, even higher levels have been found in some wild accessions (PROHENS et al. 2003), suggesting that wild species relative to pepino might be utilized for the improvement of ascorbic acid concentration in the cultivated species.

We studied the concentration of individual sugars, organic acids, and ascorbic acid in fruits of *S. muricatum*, *S. caripense*, *S. tabanoense* and interspecific hybrids. The results are expected to provide useful information for the improvement of pepino fruit quality.

# **Material and Methods**

#### Plant material

Accessions EC-37 (S. muricatum), EC-40 (S. caripense) and EC-26 (S. tabanoense), and the interspecific hybrids

EC-37×EC-40, EC-37×EC-26, and EC-40×EC-26 were used for the present study. A total of 250 plants were grown. As yield of wild *S. caripense* and *S. tabanoense*, and interspecific hybrids is usually much lower than that of *S. muricatum* (PROHENS et al. 2003), a greater number of plants of EC-40, EC-26 and interspecific hybrids was utilized to obtain enough fruits for the analyses (20 plants of EC-37, 40 of hybrids EC-37×EC-40 and EC-37×EC-26, and 50 of EC-40, EC-26 and hybrid EC-40×EC-26).

#### Growing conditions

Plants were grown in greenhouse in Valencia (Spain) during the spring-summer growing season (PROHENS et al. 2000). Plants were spaced 0.3 m within row and 1 m between rows. Temperatures ranged from 15 to 35 °C throughout the experiment. Plants were trained with vertical strings and side-shoots were removed weekly. Drip irrigation was used and nutrients were provided with the irrigation water, at a dose of 1 kg m<sup>-3</sup> of a commercial water-soluble fertilizer (15N-5P-30K plus micronutrients, BASF, Spain). Flowers of S. muricatum (EC-37) and interspecific hybrids were mechanically vibrated to improve fruit set. Because of the strict self-pollination conditions of greenhouse cultivation and the self-incompatibility of *S. caripense* and *S. taban*oense (MIONE and ÂNDERSON 1992), the tested accessions of these species were hand-pollinated twice a week with a mixture of pollen of several plants of the same accession.

#### Traits studied

Morphological and agronomic traits recorded were: yield (g plant<sup>-1</sup>), fruit length/width ratio, mean fruit weight (g), fruit colour, and number of locules. Between 20 and 60 fruits per accession (depending on the fruit size) were used for the analytical measurements and divided into three lots (6–20 fruits each). Analytical measurements were repeated three times for each lot.

Fruits were peeled, squeezed, and homogenized. Aliquots of the homogenized material were analysed for dry matter, pH, ascorbic acid, and organic acids. An additional aliquot was lyophilised for the analysis of soluble sugars.

Dry matter content (DM) was estimated by drying at 100 °C (±2 °C) to constant weight. pH was measured with a pH-meter (MicropH-2000, Crison Instrument) over a homogenized sample diluted in distilled water. Content of individual organic acids (citric, malic, oxalic, and glutamic) and ascorbic acid (AAC) were estimated by high performance liquid chromatography (HPLC) after extraction in metaphosphoric acid at 4.5 % (VázQU-EZ-ODÉRIZ et al. 1994) using a liquid chromatographer (Micrón Analítica, Madrid, Spain), supplemented with an isocratic pump (model PU-II), a sphereclone column [model ODS (2)]  $250\times4.6$  mm (5  $\mu$ ), a UV-VI detector (Thermo Separation Sectra Series UV 100), an AS-1555 automatic injector (Jasco, Japan) and software Biocrom 2000 1.0. Chromatographic conditions were: distilled water adjusted to pH 2.5-2.6 using sulphuric acid (0.01 %) as the mobile phase, and flows of  $0.9 \text{ ml min}^{-1}$ and 0.4 ml min<sup>-1</sup>, and wavelengths of 245 and 215 nm for the analysis of ascorbic acid and organics acids, respectively. Total acids (TA) were estimated as

the sum of the content of individual organic acids (TA = citric + malic + oxalic + glutamic).

Individual soluble sugars (glucose, fructose and sucrose) were analysed by HPLC after extraction of the freeze-dried sample in methanol at 80 %, followed by drying by evaporation, dissolution in water, purification with a Č18 SepPak cartridge (Waters, Mildford, MA, USA), and dissolution in the mobile phase (CÁMARA et al. 1996). The equipment used was a liquid chromatographer (Micrón Analítica), equipped with an isocratic pump (model PU-II), a  $300 \times 3.9 \text{ mm}$  (10 µ) amino bonded column (µBondapak carbohydrate, Waters), an R 401 refractometric detector (Waters), an AS-1555 automatic injector (Jasco) and software Biocrom 2000 1.0. Chromatographic conditions were: acetonitrile/water 80/20 as mobile phase and a flow of 0.9 ml min-1. Total soluble sugars (TSS) were estimated as the sum of the content of individual soluble sugars (TSS = glucose + fructose + sucrose).

# Results

# Morphological and agronomic traits

Fruits of all the accessions and hybrids were fleshy berries, often with two locule, although in the case of



Fig. 1. Fruits of the tested species: *S. muricatum* EC-37 (top center), *S. tabanoense* EC-26 (bottom left), and *S. caripense* EC-40 (bottom right). Fruits of interespecific hybrids are situated between their respective parents. Coin for size reference has a diameter of 25 mm.

EC-37 (*S. muricatum*) fruits with three or four locules were found occasionally. EC-37 had less seeds and more flesh than the wild accessions EC-26 and EC-40. Interspecific hybrids involving the cultivated species (EC-37×EC-26 and EC-37×EC-40) showed an intermediate morphology between their corresponding parents in terms of flesh and seeds, although EC-37×EC-40 was much more similar to EC-40 than to EC-37. The hybrid between the two wild species (EC-40×EC-26) showed characteristics similar to its parents. Ripe fruits of EC-37 were yellow with purple stripes, while wild accessions and interspecific hybrids were green with purple stripes (Fig. 1).

were green with purple stripes (Fig. 1). Accession EC-37 (*S. muricatum*) was the most productive, with a yield above 1000 g plant<sup>-1</sup>, while EC-26 (*S. tabanoense*) and EC-40 (*S. caripense*), gave the lowest yields (Table 1) despite periodical application of hand pollination. Yields of the interspecific hybrids involving the cultivated species ranged between 300 and 500 g plant<sup>-1</sup>. The yield of the hybrid between the wild species (EC-40×EC-26) was low, although higher than either of its parents (Table 1).

As expected, the *S. muricatum* accession had a markedly higher fruit weight than wild accessions (>140 g and  $\leq 10$  g, respectively) (Table 1). Fruit weight in the hybrids between the cultivated and the wild accessions (EC-37×EC-26 and EC-37×EC-40) was intermediate between their respective parents, although the hybrid with *S. tabanoense* produced heavier fruits than those of the hybrid with *S. caripense* (>85 g and <30 g, respectively). The shape of EC-37 fruits was rounded, with a length:width ratio close to 1, while the fruits from EC-26 and EC-40 were slightly ovate. Amazingly, fruits of interspecific hybrids were always more elongated than those of the parents, especially in the case of EC-37×EC-26, which had a length:width ratio above 1.5 (Table 1).

# Physico-chemical traits

Fruit dry matter content ranged from 6.77 to 13.79 % (Table 1). The highest DM values were found in the wild accessions and their hybrid, with values >13 %. In the case of the hybrids of *S. muricatum* with the wild accessions, a significant difference was found between them (Table 1). In particular, the hybrid with *S. caripense* (EC-37×EC-40) had a DM mean value above 12.5 %, which is intermediate between both parents, while the hybrid with *S. tabanoense* (EC-37×EC-26) had a lower DM than either parent (Table 1).

Table 1. Yield, fruit weight, shape of fruit, dry matter content of fruit (DM) and pH of fruit juice (mean $\pm$ SE) of pepino (*S. muricatum* = EC-37), wild related species (*S. tabanoense* = EC-26 and *S. caripense* = EC-40) and their interspecific hybrids.

Accession	Yield (g plant <sup>-1</sup> )	Mean fruit weight (g)	Length:width ratio	DM (%)	pН
EC-37	1162 ± 61	143.8 ± 4.4	$1.080 \pm 0.011$	$9.60 \pm 0.28$	$5.36 \pm 0.20$
EC-26	$26 \pm 4$	$10.0 \pm 0.7$	$1.235 \pm 0.012$	$13.79 \pm 2.33$	$3.87 \pm 0.08$
EC-40	$66 \pm 6$	$8.8 \pm 0.2$	$1.140 \pm 0.018$	$13.32 \pm 0.30$	$3.70 \pm 0.20$
EC-37×EC-26	$462 \pm 20$	86.7 ± 4.1	$1.542 \pm 0.024$	$6.77 \pm 0.64$	$4.74 \pm 0.03$
EC-37×EC-26	$351 \pm 18$	26.8 ± 1.4	$1.273 \pm 0.014$	$12.67 \pm 0.32$	$4.27 \pm 0.03$
EC-40×EC-26	$95 \pm 6$	$12.3 \pm 0.3$	$1.280 \pm 0.012$	$13.28 \pm 0.29$	$3.71 \pm 0.04$

Table 2. Individual and total soluble sugar	(TSS) concentration (g 100g <sup>-1</sup> )	) (mean±SE) in fruit of pepino	(S. muricatum =
EC-37), wild related species (S. tabanoense =	EC-26 and S. caripense = $EC-40$	)) and their interspecific hybrids.	

Accession	Glucose	Fructose	Sucrose	TSS
EC-37	$0.61 \pm 0.16$	$1.06 \pm 0.18$	$0.91 \pm 0.22$	$258 \pm 0.56$
EC-26	$0.01 \pm 0.10$ $0.18 \pm 0.01$	$0.13 \pm 0.01$	$0.56 \pm 0.07$	$0.86 \pm 0.06$
EC-40	$0.22 \pm 0.03$	$0.16 \pm 0.03$	$1.71 \pm 0.62$	$2.09 \pm 0.67$
EC-37×EC-26	$0.50 \pm 0.16$	$0.81 \pm 0.28$	$0.54 \pm 0.17$	$1.84 \pm 0.60$
EC-37×EC-40	$0.32 \pm 0.04$	$0.30 \pm 0.03$	$1.82 \pm 0.30$	$2.43 \pm 0.36$
EC-40×EC-26	$0.18 \pm 0.01$	$0.18 \pm 0.01$	1.47 ± 0.21	$1.82 \pm 0.21$

Table 3. Individual, total acid (TA), and ascorbic acid concentration (mg  $100g^{-1}$ ) (mean $\pm$ SE) in fruit of pepino (*S. muricatum* = EC-37), wild related species (*S. tabanoense* = EC-26 and *S. caripense* = EC-40) and their interspecific hybrids.

Accession	Citric acid	Glutamic acid	Malic acid	Oxalic acid	Total acid	Ascorbic acid	
EC 37	$228.0 \pm 43.1$	$30.6 \pm 4.6$	$97 \pm 51$	$66 \pm 0.8$	$275.7 \pm 40.8$	$118 \pm 22$	
EC-37 EC-26	$228.9 \pm 43.1$ $2297.2 \pm 65.8$	$108.4 \pm 22.3$	$306.7 \pm 14.8$	$232.4 \pm 9.5$	$273.7 \pm 40.8$ 2944.6 ± 72.8	$4.9 \pm 2.1$	
EC-40	$2037.7 \pm 81.7$	$146.4 \pm 24.8$	$249.6 \pm 17.8$	$46.4 \pm 7.0$	$2510.1 \pm 92.6$	$31.1 \pm 0.9$	
EC-37×EC-26	414.6 ± 63.6	19.9 ± 2.3	$56.6 \pm 7.7$	$53.0 \pm 15.1$	544.1 ± 75.6	$18.0 \pm 5.6$	
EC-37×EC-40	$1600.0 \pm 30.0$	$71.7 \pm 2.5$	$246.7 \pm 12.5$	$40.2 \pm 6.8$	$1958.7 \pm 43.8$	$37.2 \pm 2.2$	
EC-40×EC-26	2393.5 ± 225.2	$212.1 \pm 20.4$	$251.0 \pm 32.6$	$24.1 \pm 6.0$	$2880.8 \pm 262.1$	$29.0~\pm~2.0$	

The highest pH value was found in EC-37 (5.36), while the wild accessions EC-26, EC-40, and their hybrid showed the lowest values (3.70–3.87) (Table 1). The interspecific hybrids with EC-37 had pH values intermediate between the parents.

Significant differences among accessions were found for total soluble solids. S. muricatum (EC-37), S. caripense (EC-40) and their hybrid showed the highest levels, which ranged from 2.0 to 2.6 g  $100 \text{ g}^{-1}$  (Table 2). Although S. tabanoense (EC-26) showed the lowest values  $(<0.9 \text{ g} 100 \text{ g}^{-1})$ , the interspecific hybrids including this accession as a parent had levels greater than 1.8 g 100 g-<sup>1</sup>. Significant differences were found among accessions regarding the concentration of individual sugars. The wild accessions EC-26 and EC-40 and their hybrid EC-40×EC-26 had the lowest levels of glucose and fructose, while the highest levels corresponded to EC-37 and the hybrid EC-37×EC-26. The highest concentrations of sucrose were found in EC-40 and the hybrids including this accession as a parent, while EC-26 and EC-37×EC-26 showed the lowest values (Table 2).

Two different patterns were found with respect the relative concentrations of the different sugars. In particular, EC-37 and EC-37×EC-26 had similar profiles of the three sugars, and fructose was the sugar with the highest concentration (around 40 % of TSS). In the wild accessions and the other hybrids, sucrose was the predominant sugar (65-80 % of TSS) (Fig. 2).

Important differences were found also in the total concentration of acids. EC-37 had the lowest values (<300 mg 100 g<sup>-1</sup>), while the wild accessions and their interspecific hybrid showed levels >2500 mg 100 g<sup>-1</sup>. The interspecific hybrids involving *S. muricatum* had TA values intermediate between those of the respective parents, while TA of the hybrid with *S. caripense* 

(EC-37×EC-40) was four times higher than those of the hybrid with *S. tabanoense* (EC-37×EC-26) (Table 3).

Citric acid was the most abundant organic acid (accounting for more than 75 % of TA) in all the materials studied (Fig. 3), and ranged from 228.9 mg 100 g<sup>-1</sup> in EC-37 to 2393.5 mg 100 g<sup>-1</sup> in EC-40×EC-26 (Table 3). Malic acid ranked second to citric acid in all



Fig. 2. Relative concentrations (%) of glucose, fructose and sucrose in pepino (*S. muricatum* = EC-37), wild related species (*S. tabanoense* = EC-26 and *S. caripense* = EC-40) and their interspecific hybrids.

materials, with the exception of EC-37, in which glutamic acid was the acid with the second highest concentration (Fig. 3; Table 3). In general, oxalic acid was the least important of the organic acids, with levels ranging from  $6.6 \text{ mg } 100 \text{ g}^{-1}$  in EC-37 to 232.4 mg 100 g<sup>-1</sup> in EC-26 (Table 3).

The concentration of ascorbic acid was close to  $12 \text{ mg } 100 \text{ g}^{-1}$  in EC-37, while in EC-40 it was more than three times higher. On the contrary, *S. tabanoense* accession EC-26 showed very low AAC levels (lower than 5 mg 100 g<sup>-1</sup>). Interspecific hybrids had an AAC higher than the mean value found in their parents, especially in the case of hybrids including EC-37 as a parent (Table 3).

#### Correlations between traits

DM was negatively correlated to pH, glucose, and fructose; and positively to TA and to citric, glutamic and malic acids. No significant correlation was found between DM and TSS (Table 4). All individual sugars were positively correlated to TSS. Glucose and fructose were correlated, but no correlation was found between sucrose and either glucose or fructose

Each individual acid, with the exception of oxalic acid, was positively correlated to the concentration of the other organic acid measured, as well as with TA. PH was negatively correlated to TA and to all individual acids, with the exception of oxalic acid.

Fructose and glucose were positively correlated to pH and negatively to citric, glutamic, and malic acid. On the contrary, sucrose was only correlated (positively) to glutamic acid. Oxalic acid was found to be negatively correlated to TSS. In the case of AAC, only two significant correlations were found (positive with sucrose and negative with oxalic acid) (Table 4).

#### Discussion

The results obtained indicate that wild species *S. caripense* and *S. tabanoense* may be utilized to improve the composition of pepino fruit, especially by increasing the

acidity and ascorbic acid content. Although yield and fruit size of interspecific hybrids involving the cultivated species *S. muricatum* were lower than those obtained by accessions of the latter, recovery of yield and fruit size typical to the domesticate can be obtained after a few backcross generations (HEISER 1964; PROHENS et al. 2003). Although fruit colour in the hybrids was similar to that of wild accessions (green skin with purple or dark-green stripes), PROHENS et al. (2003) reported that individuals with yellow skin, characteristic of *S. muricatum*, may be found in the first backcross generation, suggesting that this trait is under the control of a single gene, for which alleles from the wild species are dominant.



Fig. 3. Relative concentrations (%) of citric, malic, glutamic and oxalic acids in pepino (*S. muricatum* = EC-37), wild related species (*S. tabanoense* = EC-26 and *S. caripense* = EC-40) and their interspecific hybrids.

Table 4. Correlations between traits related to fruit composition (DM = % dry matter content, TSS = total soluble solids, TA = total acid concentration, AAC = ascorbic acid concentration).

	pН	Glucose	Fructose	Sucrose	TSS	Citric	Glutamic	Malic	Oxalic	ТА	AAC
DM pH Glucose Fructose Sucrose TSS Citric		-0.66** 0.71**	-0.74*** 0.82*** 0.96**	0.38 <sup>NS</sup> -0.38 <sup>NS</sup> -0.02 <sup>NS</sup> -0.19 <sup>NS</sup>	-0.21 <sup>NS</sup> 0.26 <sup>NS</sup> 0.68* 0.56* 0.70**	0.86*** -0.93*** -0.76*** -0.84*** 0.32NS -0.33NS	0.68** -0.82*** -0.61** -0.65** 0.49* -0.07NS 0.82***	0.80*** -0.89*** -0.70** -0.82*** 0.34NS -0.29NS 0.95***	0.32 <sup>NS</sup> -0.36 <sup>NS</sup> -0.31 <sup>NS</sup> -0.34 <sup>NS</sup> -0.38 <sup>NS</sup> -0.55* 0.43 <sup>NS</sup>	0.85*** -0.93*** -0.75*** -0.84*** 0.30NS -0.34NS 1.00***	0.20 <sup>NS</sup> -0.33 <sup>NS</sup> -0.11 <sup>NS</sup> -0.22 <sup>NS</sup> 0.70** 0.42 <sup>NS</sup> 0.25 <sup>NS</sup>
Glutamic Malic								0.66**	$0.05^{NS}$ $0.52^{*}$	$0.80^{***}$ $0.96^{***}$	0.37 <sup>NS</sup> 0.28 <sup>NS</sup>
Oxalic TA										0.48*	-0.51* 0.22 <sup>NS</sup>

NS, \*, \*\*, \*\*\* Nonsignificant at P<0.05 and significant at P<0.05, 0.01, and 0.001, respectively.

Wild accessions had much higher DM, individual acids, and TA concentrations than the cultivated species, and similar (EC-40) or lower (EC-26) concentrations of sugars. Pepino fruits are considered subacid (NA-TIONAL RESEARCH COUNCIL 1989) and a slight increase in acidity might contribute to increase fruit quality. Due to their acidity, wild species could be a useful source of variation for improving the flavour intensity of pepino. In this sense, hybrids between the cultivated and wild accessions had acidity values intermediate between the parents, with a higher proximity to the values of the cultivated species, especially in the case of EC-37×EC-26. These results suggest that the intense acidity of wild species can be reduced to levels slightly higher than those of the cultivated species in a few backcrosses after interspecific hybridisation.

Regarding the proportions of sugars, two different patterns were found among the materials studied. As occurs in tomato (YELLE et al. 1991; STOMMEL and HAYNES 1993), sucrose was predominant in the wild EC-37×EC-40 hybrids accessions and and EC-40×EC-26, while the cultivated accession EC-37 and the hybrid EC-37×EC-26 contained significantly less sucrose with similar levels of the three individual sugars. Such differences may be attributed to differences in pH, which affects the activity of acid invertase, the enzyme that hydrolyses sucrose into fructose and glucose. The maximum activity of this enzyme in muskmelon and tomato occurs at a pH around 5.0 (IWATSU-BO et al. 1992; BUCHELI and DEVAUD 1994), which might explain why EC-37 and EC-37×EC-26, which had pH levels close to 5.0, showed lower levels of sucrose than the other accessions. The positive correlations found between pH and the levels of glucose and fructose supports this hypothesis. Further genetic differences affecting the invertase enzyme activity could also be involved in the different patterns of sugar accumulation among the tested species and their hybrids. If the accumulation of sucrose in pepino is determined either by pH, or by genes directly controlling the invertase activity, or by both, has an implication in breeding for fruit quality. In the first case it would not be possible to get high sucrose concentrations with the characteristic pH of pepino. In the second case, higher sucrose concentrations in pepino may be obtained if the acid invertase genes from the wild species were introgressed into pepino. The obtained data suggests that the differences in sucrose accumulation in pepino fruit are more likely due to pH differences. If the differences had a genetic base, it probably would involve a complex genetic system, in which genes from S. caripense would be dominant over genes from S. muricatum and S. tabanoense and, also, genes from S. muricatum would be dominant over genes from S. tabanoense.

Citric acid was the predominant acid not only in pepino, which is in agreement with REDGWELL and TURN-ER (1986) and HUYSKENS-KEIL et al. (2000), but also in wild accessions and the interspecific hybrids. The relatively high proportion of citric acid in the composition of pepino, together with C9 aldehydes in the volatile fraction, its flavour may make resemble that of muskmelon (SHIOTA et al. 1988; HUYSKENS-KEIL et al. 2000; BURGER et al. 2003).

AAC levels found for *S. muricatum* in this work are lower than those reported by REDGWELL and TURNER

(1986), who obtained values ranging from 48 to 68 mg 100g<sup>-1</sup>. Dissimilarities in plant material and environmental conditions during cultivation could account for such differences. Some authors (SÁNCHEZ et al. 2000; RODRÍGUEZ-BURRUEZO et al. 2002) reported that the higher the temperature during ripening, the lower the AAC of pepino fruits. Thus, ripening of fruits in summer as a consequence of cultivation in the spring-summer growing season might be related to the comparatively low levels of AAC recorded in this experiment. Nonetheless, the higher values of AAC in EC-40 and its hybrid with the cultivated species under identical environmental conditions indicate that *S. caripense* may be of interest for the improvement of this trait in pepino.

In conclusion, the wild species show a fruit composition different to that of the cultivated species. These differences, particularly the high acidity of EC-26 and EC-40, and the high AAC of EC-40, may be utilized to modify the sugars/acids ratio and to increase the ascorbic acid content of pepino. In addition, the significant differences in the relative concentration of sugars between the tested species and hybrids indicate that pepino and wild relatives may be utilized in studies of the physiology of sugar accumulation.

# References

- ANDERSON, G.J., R.K. JANSEN and Y. KIM 1996: The origin and relationships of the pepino, *Solanum muricatum* (Solanaceae) DNA restriction evidence. Econ. Bot. 50, 369–380.
- BURGER, Y., U. SA'AR, A. DISTELFELD, N. KATZIR, Y. YE-SELSON, S. SHEN and A.A. SCHAFFER 2003: Development of sweet melon (*Cucumis melo*) genotypes combining high sucrose and organic acid content. J. Amer. Soc. Hort. Sci. **128**, 537–540.
- BUCHELI, P. and S. DEVAUD 1994: Sugar accumulation in tomato and partial purification of buffer-insoluble invertase. Phytochemistry **36**, 837–841.
- CÁMARA, M.M., C. DÍEZ and M.E. TORIJA 1996: Free sugars determination by HPLC in pineapple products. Z. Lebensm. Unters. Forsch. 202, 233–237.
- EL-ZEFTAWI, B.M., L. BROHIER, L. DOOLEY, F.H. GOU-BRAN, R. HOLMES and B. SCOTT 1988: Some maturity indices for tamarillo and pepino fruits. J. Hort. Sci. **63**, 163–169.
- HEISER, C.B. 1964: Origin and variability of the pepino (*Solanum muricatum*): a preliminary report. Baileya **12**, 151–158.
- HEISER, C.B. 1985: Of plants and people. University of Oklahoma Press, Norman, Oklahoma, USA.
- HUYSKENS-KEIL, S., H.P. WIDAYAT, P. LÜDDERS, M. SCHREINER and P. PETERS 2000: Physiological changes of pepino (*Solanum muricatum* Ait.) during maturation and ripening. Acta Hort. **531**, 251–256.
- IWATSUBO, T., H. NAKAGAWA, N. OGURA, T. HIRABA-YASHI and T. SATO 1992: Acid invertase of melon fruits: immunochemical detection of acid invertases. Plant Cell Physiol. **33**, 1127–1133.
- KALLOO, G. and B.O. BERGH 1993: Genetic improvement of vegetable crops. Pergamon Press, Oxford, UK.
- KILCAST, D., M.O. PORTMANN and B.E. BYRNE 2000: Sweetness of bulk sweeteners in aqueous solution in the presence of salts. Food Chem. **70**, 1–8.

- LESTER, R.N. 1991: Evolutionary relationships of tomato, potato, pepino and wild species of *Lycopersicon* and *Solanum*. In: HAWKES, J.G., R.N. LESTER, M. NEE and N. ESTRADA (eds): *Solanaceae* III: taxonomy, chemistry, evolution. Royal Botanic Gardens at Kew and Linnean Society of London, London, pp. 283–301.
- MIONE, T. and G.J. ANDERSON 1992: Pollen-ovule ratios and breeding system evolution in *Solanum* section *Basarthrum (Solanaceae)*. Amer. J. Bot. **79**, 279–287.
- NATIONAL RESEARCH COUNCIL 1989: Lost crops of the incas: little-known plants of the Andes with promise for worldwide cultivation. National Academy Press, Washington DC.
- NUEZ, F., R. MORALES, J. PROHENS, P. FERNÁNDEZ DE CÓRDOVA, S. SOLER, E. VALDIVIESO and V. SOLÓRZA-NO 1999: Germplasm of *Solanaceae* horticultural crops in the south of Ecuador. Plant Genetic Resources Newsletter **120**, 44–47.
- O'NEIL, M.J., A. SMITH, P.E. HECKELMAN, J.R. OBEN-CHAIN, J.A.R. GALLIPEAU, M.A. D'ARECCA and S. BU-DAVARI 2001: The Merck index – an encyclopedia of chemicals, drugs, and biologicals. 13<sup>th</sup> edition. Merck Research Laboratories, Whitehouse Stations, New Jersev.
- PROHENS, J., J.J. RUIZ and F. NUEZ 2000: Growing cycles for a new crop, the pepino, in the Spanish Mediterranean. Acta Hort. 523, 53–60.
- PROHENS, J., G.J. ANDERSON, A. RODRÍGUEZ-BURRUE-ZO and F. NUEZ 2003: Exploiting wild species for the genetic improvement of the pepino (*Solanum muricatum*). J. Appl. Bot. **77**, 21–27.
- PRONO-WIDAYAT, H., M. SCHREINER, S. HUYSKENS-KEIL and P. LÜDDERS 2003: Effect of ripening and storage temperature on postharvest quality of pepino (*Solanum muricatum* Ait.). Int. J. Food Agr. Environment **1**, 35–41.
- REDGWELL, R.J. and N.A. TURNER 1986: Pepino (*Solanum muricatum*): Chemical composition of ripe fruit. J. Sci. Food Agr. **37**, 1217–1222.
- RODRÍGUEZ-BURRUEZO, A., J. PROHENS and F. NUEZ 2002: Genetic analysis of quantitative traits in pepino in two growing systems. J. Amer. Soc. Hort. Sci. **127**, 271–278.
- ROSELLÓ, S., L. GALIANA-BALAGUER, J.M. HERRE-RO-MARTÍNEZ, A. MAQUIEIRA and F. NUEZ 2002: Simultaneous quantification of the main organic acids

and carbohydrates involved in tomato flavour using capillary zone electrophoresis. J. Sci. Food Agr. 82, 1101–1106.

- SÁNCHEZ, M., M. CÁMARA, J. PROHENS, J.J. RUIZ, E. TOR-IJA and F. NUEZ 2000: Variation in carbohydrate content during ripening in two clones of pepino. J. Sci. Food Agr. **80**, 1985–1991.
- SCHAFFER, A.A., I. RYLSKI and M. FOGELMAN 1989: Carbohydrate content and sucrose metabolism in developing *Solanum muricatum* fruits. Phytochemistry **28**, 737–739.
- SHIOTA, H., H. YOUNG, V.J. PATERSON and M. IRIE 1988: Volatile aroma constituents of pepino fruit. J. Sci. Food Agr. **43**, 343–354.
- STEVENS, M.A., A.A. KADER, M. ALBRIGHT-HOLTON and M. ALGAZI 1977: Genotypic variation for flavour and composition in fresh market tomatoes. J. Amer. Soc. Hort. Sci. **97**, 655–658.
- STOMMEL, J.R. and K.G. HAYNES 1993: Genetic control of fruit sugar accumulation in a *Lycopersicon esculentum* X L. hirsutum cross. J. Amer. Soc. Hort. Sci. 118, 859– 863.
- VÁZQUEZ-ODÉRIZ, M.L., M.E. VÁZQUEZ-BLANCO, J. LÓPEZ-HERNÁNDEZ, J. SIMAL-LOZANO and M.A. ROMERO-RODRÍGUEZ 1994: Simultaneous determination of organic acids and vitamin C in green beans by liquid chromatography. J. AOAC Int. 77, 1056–1059.
- liquid chromatography. J. AOAC Int. **77**, 1056–1059. WELLES, G.W.H. 1992: Experiences with growing and consumer appreciation of pepino fruits (*Solanum muricatum*) in the Netherlands. Acta Hort. **318**, 211–212.
- YELLE, S., R.T. CHETELAT, M. DORAIS, J.W. DE VERNA and A.B. BENNETT 1991: Sink metabolism in tomato fruit. IV. Genetic and biochemical analysis of sucrose accumulation. Plant Physiol. **95**, 1026–1035.

Received April 05, 2004 / Accepted February 21, 2005

Addresses of authors: Jaime Prohens, Adrián Rodríguez-Burruezo, and Fernando Nuez (corresponding author), Centro de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV), Universidad Politécnica de Valencia, Camino de Vera 14, 46022, Valencia, Spain and M. Cortes Sánchez, Montaña Cámara, and Esperanza Torija, Departamento de Nutrición y Bromatología II, Facultad de Farmacia, Universidad Complutense de Madrid, Plaza Ramón y Cajal s/n, 28040, Madrid, Spain, e-mail: fnuez@btc.upv.es.