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Characterisation of Tomato Germplasm by Pericarp Protein Profiles and Morphologic and Biochemical Fruit Traits

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ABSTRACT

A diversity of genetic materials contained in traditional varieties, modern cultivars and wild species contributes to plant genetic resources. The aim of this work was to characterise different tomato (*Solanum* spp.) genotypes by pericarp protein profiles and morphologic and biochemical fruit traits. Three accessions of the wild taxa *S. pimpinellifolium* (LA722, LA2181 and LA1246) and *S. lycopersicum* var. *cerasiforme* (LA1673, LA1385 and LA1320), four cultivated genotypes of *S. lycopersicum* ('Caimanta', 'CARO', 'UCO', 'NOR') and nine RILs (L1, L2, L4, L5, L6, L7, L8, L9, L18) obtained the interspecific cross (LA722 x 'Caimanta') were studied. Highly significant differences were found between these genotypes for all traits and the cluster analysis allowed to separate cultivated genotypes from the others. Pericarp protein profiles were obtained at two ripening stages (mature green: GM and red ripe: RR) to classify these genotypes. At MG, the percentage of polymorphism between the cultivated genotypes was 81%, while among the wild genotypes it was 94.6%. The highest polymorphism (97.3%) was observed in the RILs. The most frequent band (0.79) was that of 97 kDa present in all genotypes excepting L8, LA1320, LA1673, and 'CARO'. At RR both cultivated genotypes and wild accession showed 92% polymorphic bands. Polymorphism among RILs was 94.6%. Again, a 97 kDa band was detected in all genotypes except for L18 while a 56 kDa band was observed in all genotypes with the exception of 'NOR' and L6. Genotype clustering by pericarp protein profile was different to that obtained by morphological and biochemical fruit traits.

Keywords: cluster analysis, genetic diversity, plant breeding, *Solanum* section *Lycopersicon*

INTRODUCTION

Current plant breeding provokes a loss of genetic variability, which could lead to crop genetic uniformity. As a consequence of the domestication process and breeding during recent years, a great loss of variability was observed because of the disappearance of complete genotypes, individual loci, or even alleles from the same gene (Babeli and Mazzucato 2008). This fact can be reverted by the utilization of wild species germplasm in breeding programmes. Wild species of the section *Lycopersicon* in the genus *Solanum* represent an important source of genetic variability, being a potential genetic resource to developed varieties in breeding programmes (Schauer *et al*. 2006). Wild relatives of *S. lycopersicum* have been used to introduce genes for insect and disease resistance as well as tolerance to adverse environment conditions such as drought and/or salinity (Zhang *et al*. 2002) but these species also usually have variability of fruit quality traits such as flavor, aroma, color, and texture. Under natural conditions the ability of wild species to maintain fruit quality over an extended period of time could be an adaptive strategy to attract herbivores and to ensure seed dispersion (Nakazato *et al*. 2008). Several authors studied the potentiality of improving tomato fruit quality by means of incorporating wild germplasm (Zorzoli *et al*. 1998; Pratta *et al*. 2000; Gur and Zamir 2004; Garg *et al*. 2008). Rodríguez *et al*. (2006a) obtained 17 Recombinant Inbred Lines (RILs) of tomato from an interspecific cross between *S. lycopersicum* cv. 'Caimanta' and *S. pimpinellifolium* accession LA722 and they found that some of these RILs had longer shelf life and higher soluble solid content than the wild parent (Rodríguez *et al*. 2006b).

Most fruit quality traits are related to tomato ripening. This process is the result of highly synchronized biochemical and physiological changes that occur during a relatively short period. These changes allow different ripening stages to be distinguished (Giovannoni 2004). According to Aivalakis and Katinakis (2008), protein synthesis appears to be an essential component of these ripening stages. Therefore, SDS-PAGE of proteins from individuals at different ripening stages might be able to detect variations in gene expression involved in this biological process (Zhang and Riechers 2008). Protein profiles have been successfully used on several species as a molecular marker (De Luca *et al*. 2000; Garello *et al*. 2000; Padmavatti *et al*. 2001; Rodríguez *et al*. 2008). Even though they are less polymorphic than DNA markers, they provide quick and easy information to characterise genotypes. The aim of this work was to characterise different tomato genotypes by pericarp protein profiles and morphologic and biochemical fruit traits.

MATERIALS AND METHODS

Plant material

Three accessions of the wild taxa *S. pimpinellifolium* (LA722, LA2181 and LA1246) and *S. lycopersicum* var. *cerasiforme* (LA1673, LA1385 and LA1320), four cultivated genotypes of *S. lycopersicum* ('Caimanta', 'CARO', 'UCO', 'NOR') and nine RILs (L1, L2, L4, L5, L6, L7, L8, L9, L18) obtained by Rodríguez *et al.* (2006a) were studied. The wild genotypes were provided by the Tomato Genetic Resources Center (TGRC) from the University of California, Davis (USA) and the cultivated genotypes were provided by EEA INTA (Estación Experimental Agropecuaria – Instituto Nacional de Tecnología Agropecuaria, Argentina). These genotypes were cultivated under greenhouse during spring season

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(August-December), at the field station "José F. Villarino" (Facultad de Ciencias Agrarias UNR, Zavalla, Argentina, 33° South Latitude and 61° West Longitude) in a completely randomized design. Nine to 10 plants by genotype were sowed, the total number of plants being 181. Previous to the transplantation, the soil (a typical Argiudol) was fertilized with poultry grit. The plants were watered twice a week, levels of irrigation that were sufficient to avoid water stress during the plants growing period. Mean temperature was 20 ± 3 ºC, mean light intensity was 700 µmol.s^{-1}.m^{-2} and mean relative humidity was 60%.

Morphologic and biochemical fruit traits

The following fruit traits were evaluated in 10 fruits per plant at the breaker stage (Giovannoni 2004): diameter (D, in cm), height (H, in cm), shape (Sh, H/D ratio), weight (W, in g), and shelf life (SL, in days) defined as the time period until a water-soaked spot appeared on the surface of the fruit stored at 25 ± 2°C (Schuelter *et al*. 2002; Garg *et al*. 2008). The fruits were examined three times a week and commercially unacceptable fruits (i.e. showing wrinkling and excessive softening) were discarded. A total of 1579 fruits were evaluated. At the Red Ripe stage (RR) (Giovannoni 2004) the following traits of over 900 fruits were also evaluated: locule number (LN), pericarp thickness (T, in mm), firmness (F) and color (C) this being trait measured by both the reflectance percentage (L) and the (a/b) ratio. The parameters of L, a (absorbance at 540 ηm) and b (absorbency at 675 ηm) were determined with a Chromameter CR300 (three data points equally spaced over the equatorial region of each fruit). Firmness was measured in two opposite areas over the equatorial region of three fruits per genotype with a durometer Shore A Durofel using a 0.10 cm^2-area plunger. The F and C traits were measured on five fruits per plant.

The other traits related to internal quality were obtained in three samples of 3 to 8 fruits per plant depending of fruit size. They were: soluble solid content (SS, in °Brix) measured by a hand refractometer, titrable acidity: (At, in g of citric acid/100 g of homogenized fruit juice) calculated from the volume of 0.1 N NaOH necessary for turning the pH of 2 g of juice dissolved in 20 ml of distillated water to 8.1, and the pH (Garg *et al.* 2008).

Protein profiles

Three fruits by genotype were harvested at two ripening stages: Mature Green (MG) and Red Ripe (RR). Proteins were extracted from the pericarp following the method of Hurkman and Tanaka (1986) with modifications. Briefly, 1 g of tissue (tomato pericarp) was first ground with a mortar and pestle device in liquid nitrogen. Then it was homogenised and thoroughly mixed in the presence of 1 ml of extraction buffer (100 mM Tris/HCl pH 8.0, 1 mM EDTA, 1 mM PMSF, 2% (v/v) β-mercaptoethanol) and 4 ml of phenol saturated with 100 mM Tris buffer (pH 8.0), and then centrifuged for 15 min at 5000 rpm at 4°C. The phenolic phase was removed, re-extracted with one volume of aqueous buffer and mixed with four volumes of 0.1 M ammonium acetate in methanol, and incubated overnight at -20°C. Proteins were precipitated by centrifugation for 20 min at 5000 rpm at 4°C. The precipitated fraction was washed twice with 0.1 M ammonium acetate in methanol, followed by a cold acetone (80%, v/v) wash, and then dried at room temperature. Finally, the dried residue was resuspended in SDS buffer (25 mM Tris pH 6.8, 1% (w/v) SDS, 10% (v/v) glycerol, 5% (v/v) β-mercaptoethanol, and 0.002% (w/v) bromophenol blue, and boiled for 2 min. Electrophoresis was run in a Mini-PROTEAN II (Bio-Rad, California, USA) apparatus at 35 mA during 1.5 h. An equal amount of protein (30 µg) was loaded into each well. Proteins ranging from 25 to 105 kDa were electrophoresed in a stacking gel (4% polyacrylamide) followed by a separating gel (10% polyacrylamide). Running calibration proteins (ranging from 30 to 97 kDa, GE Healthcare®) were used as molecular mass marker. Proteins were stained with 0.1% Coomasie Brilliant Blue R-250 solution.

Data analysis

The normal distribution of morphologic and biochemical traits was verified by the Shapiro-Wilk (1965) test. The W, H, D, T, and F traits were transformed with the log 10 function. Mean values for these and other traits were compared by ANOVA and the differences among genotypes were estimated by LSD Fisher's test (Sokal and Rolhf 1969). For the L and LN traits the Kruskal-Wallis test was used. A multivariate analysis of clusters was applied for classifying genotypes according to the 13 morphological and biochemical traits. Average Euclidean distances and Ward's method (1963) were used as fusion criterion. Polyacrylamide gels were analyzed by quantifying the total number of polypeptides and by calculating the percentage of polymorphic polypeptides at each stage. The presence and absence of protein bands was evaluated in each genotype at each ripening stage. Finally, other two-cluster analyses were performed using Ward's method (1963) with Jaccard (1901) distance including protein profiles at the MG and RR stages. This analysis was carried out with software INFOSTAT Version 1.0.

Table 1 Mean values and standard error for the fruit traits evaluated in cultivated, wild and *RILs* genotypes.

Genotypes	Morphologic and biochemical fruit traits												
	D	**H**	**Sh**	**LN**	**T**	**L**	**a/b**	**F**	**pH**	**At**	**W**	**SL**	**SS**
CAI	5.2 ± 0.1	4.5 ± 0.1	0.9 ± 0.1	4	n/d	42.4 ± 0.8	0.8 ± 0.1	60.0 ± 4.0	4.6 ± 0.1	0.3 ± 0.1	72.0 ± 5.0	18.0 ± 3.0	5.0 ± 0.2
CARO	4.3 ± 0.2	4.7 ± 0.2	1.1 ± 0.1	2	5.5 ± 1.0	39.2 ± 0.4	1.0 ± 0.1	54.4 ± 0.5	4.5 ± 0.1	0.3 ± 0.1	52.0 ± 7.0	16.0 ± 2.0	4.6 ± 0.2
UCO	6.6 ± 0.3	4.6 ± 0.2	0.7 ± 0.1	6	4.1 ± 0.4	42.6 ± 0.9	0.8 ± 0.1	45.0 ± 2.0	4.4 ± 0.1	0.2 ± 0.1	126.0 ± 13.0	13.0 ± 1.0	4.1 ± 0.2
NOR	4.1 ± 0.3	3.6 ± 0.2	0.9 ± 0.1	2	4.3 ± 0.2	48.0 ± 1.0	0.5 ± 0.1	52.0 ± 2.0	4.7 ± 0.1	0.2 ± 0.1	39.0 ± 6.0	44.0 ± 4.0	4.9 ± 0.3
LA1320	3.0 ± 0.1	2.4 ± 0.1	0.8 ± 0.1	3	2.7 ± 0.2	40.1 ± 0.4	1.0 ± 0.1	49.0 ± 2.0	5.1 ± 0.1	0.3 ± 0.1	14.0 ± 2.0	19.0 ± 1.0	4.8 ± 0.1
LA1385	1.5 ± 0.1	1.3 ± 0.1	0.9 ± 0.1	2	0.5 ± 0.1	39.7 ± 0.4	1.3 ± 0.1	44.0 ± 1.0	5.3 ± 0.2	0.3 ± 0.1	1.9 ± 0.2	13.0 ± 1.0	7.1 ± 0.1
LA1673	1.6 ± 0.1	2.6 ± 0.1	1.0 ± 0.1	2	0.7 ± 0.1	39.0 ± 6.0	1.1 ± 0.2	46.0 ± 2.0	5.1 ± 0.1	0.2 ± 0.1	2.7 ± 0.2	15.0 ± 1.0	5.4 ± 0.1
LA1246	1.3 ± 0.1	1.2 ± 0.1	0.9 ± 0.1	n/d	n/d	37.6 ± 0.1	1.4 ± 0.1	n/d	n/d	n/d	1.2 ± 0.2	16.0 ± 3.0	n/d
LA2181	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	n/d	n/d	40.0 ± 0.1	1.3 ± 0.1	43.5 ± 0.1	n/d	n/d	0.7 ± 0.1	11.0 ± 1.0	n/d
LA722	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	2	0.5 ± 0.1	40.6 ± 0.9	1.3 ± 0.1	56.0 ± 2.0	5.1 ± 0.3	0.2 ± 0.1	1.1 ± 0.1	16.0 ± 1.0	7.0 ± 1.0
L1	3.5 ± 0.1	2.8 ± 0.2	0.8 ± 0.1	3	2.1 ± 0.3	38.5 ± 0.8	1.1 ± 0.1	55.0 ± 2.0	5.1 ± 0.1	0.2 ± 0.1	21.0 ± 2.0	26.0 ± 2.0	6.0 ± 0.1
L2	2.7 ± 0.1	2.2 ± 0.1	0.8 ± 0.1	3	2.6 ± 0.4	39.8 ± 0.2	1.3 ± 0.1	56.0 ± 2.0	5.2 ± 0.1	0.3 ± 0.1	10.0 ± 1.0	22.0 ± 1.0	7.4 ± 0.3
L4	2.4 ± 0.1	2.2 ± 0.1	0.9 ± 0.1	2	1.5 ± 0.1	37.9 ± 0.2	1.2 ± 0.1	46.0 ± 2.0	5.1 ± 0.1	0.3 ± 0.1	9.0 ± 1.0	22.0 ± 2.0	6.9 ± 0.4
L5	2.1 ± 0.1	1.9 ± 0.1	0.9 ± 0.1	2	1.8 ± 0.1	37.9 ± 0.2	1.3 ± 0.1	43.0 ± 3.0	4.7 ± 0.4	0.3 ± 0.1	6.0 ± 1.0	15.0 ± 1.0	8.1 ± 0.3
L6	1.5 ± 0.1	1.4 ± 0.1	0.9 ± 0.1	2	1.0 ± 0.1	38.2 ± 0.3	1.5 ± 0.1	46.0 ± 2.0	5.0 ± 0.2	0.4 ± 0.1	1.9 ± 0.2	21.0 ± 1.0	9.2 ± 0.5
L7	1.8 ± 0.1	1.8 ± 0.1	1.0 ± 0.1	2	1.4 ± 0.1	39.7 ± 0.9	1.1 ± 0.1	48.0 ± 4.0	4.9 ± 0.2	0.3 ± 0.1	3.9 ± 0.7	18.0 ± 1.0	6.1 ± 0.5
L8	1.3 ± 0.1	1.3 ± 0.1	1.0 ± 0.1	2	0.7 ± 0.1	39.5 ± 0.4	1.3 ± 0.1	54.0 ± 1.0	4.6 ± 0.1	0.4 ± 0.1	1.4 ± 0.1	19.0 ± 1.0	9.7 ± 0.7
L9	1.5 ± 0.1	1.4 ± 0.1	0.9 ± 0.1	2	0.6 ± 0.1	40.1 ± 0.4	1.2 ± 0.1	52.0 ± 2.0	4.6 ± 0.1	0.4 ± 0.1	2.1 ± 0.4	15.0 ± 1.0	8.8 ± 0.7
L18	2.5 ± 0.1	2.6 ± 0.1	1.0 ± 0.1	3	1.6 ± 0.1	38.4 ± 0.2	1.3 ± 0.1	48.0 ± 2.0	5.2 ± 0.1	0.2 ± 0.1	10.0 ± 1.0	21.0 ± 2.0	8.4 ± 0.1
F/χ^2	132.4**	131.4**	23.0**	42.1**	41.4**	79.5**	10.5**	4.2**	4.7**	7.3**	131.5**	10.1**	24.7**
LSD	0.06	0.06	0.05		0.15		0.21	0.06	0.37	0.74	0.17	0.12	1.1

D: diameter (cm); **H**: height (cm); **Sh**: shape; **LN**: locule number; **T**: pericarp thickness (mm); **L** and **a/b**: color parameters; **F**: firmness; **pH**: hydrogen potential, **At**: titrable acidity (g of citric acid/100 g of homogenized fruit juice); **W**: weight; **SL**: shelf life; **SS**: soluble solid content (°Brix); **n/d**: no data. **F:** ANOVA values**; χ^2:** Chi- Square values; **LSD**: Least Square Difference values; **$p \leq 0.01$

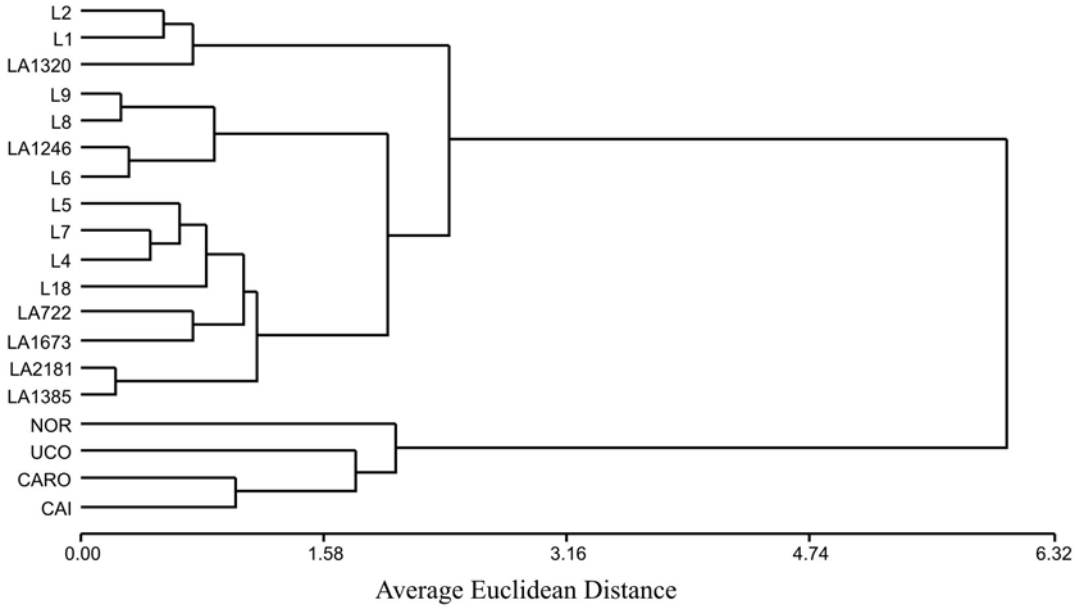

Fig. 1 Dendrogram for the 13 evaluated fruit traits in all genotypes according to Ward's method.

RESULTS

Morphological and biochemical fruit traits

Table 1 shows the mean values for all traits in each genotype. Highly significant differences were found among genotypes for all traits. Moreover, within any taxonomic group significant differences could also be observed for some traits. The cultivated genotypes ('Caimanta', 'NOR', 'UCO' and 'CARO') are outlined because they had higher H and D values than the wild taxa and the RILs. 'CARO' had the greatest shape value, contrasting with 'UCO' that had the lowest. Cultivated genotypes also had higher values for W, and wild species had the lowest values but RILs had intermediate values, closer to wild accessions. The highest and lowest values for the a/b index were observed in wild accessions and 'NOR' genotype, respectively. The acidity values varied between 0.2 and 0.4, the highest value for pH being in accession LA1385 and the lowest in 'CARO'. As expected, the greatest LN value was found in those genotypes with higher fruit mass whereas genotypes with small fruits had lower LN values. The wild taxa had F values lower than the cultivated species. 'NOR' had the longest shelf life (44 days), this being 13 days for 'UCO', although *S. pimpinellifolium* LA2181 had the lowest value (11 days). The RILs showed an intermediate value between cultivated and wild genotypes for this trait. The wild taxa had higher SS ranked among 5.4 to 7.1 °Brix, though the RILs had the highest values (7.4 to 9.6 °Brix). 'CARO' and 'UCO' had the lowest SS, 3.4 and 4.1 °Brix, respectively.

Fig. 1 shows a dendrogram of the cluster analysis including the 13 morphological and biochemical fruit traits. The cophenetic correlation was 0.57 and the average Euclidean distances ranged from 0.23 to 6.02. This multivariate analysis, which included phenotypic data, discriminated genotypes into two groups, the most important traits being D, H, W, pH, LN, T and fruit color (a/b). Wild accessions and RILs were included in the same group while the other group was only composed of cultivated genotypes.

Protein profiles

The protein profiles obtained for each genotype at both ripening stages of fruits allowed a total of 37 bands that had high polymorphism among genotypes to be detected. These polymorphisms were higher at the MG than at the RR stage. The monomorphic bands among different groups of genotypes in each ripening stage are shown in **Table 2**.

Table 2 The monomorphic bands found at two ripening stages: Mature Green (MG) and Red Ripe (RR) among the different groups of genotypes.

Group of genotypes	Monomorphic bands (kDa)	
	MG	**RR**
Cultivated	93, 88, 56, 43, 36, 28	97, 45, 39
RILs	30	66, 30
Wild species	47, 28	97, 56, 47

At the MG stage, the percentage of polymorphism between the cultivated genotypes was 81%, while among the wild genotypes it was 94.6%. However, the highest polymorphism (97.3%) was observed in the RILs. The most frequent band (0.79) was that of 97 kDa present in all genotypes excepting L8, LA1320, LA1673, and 'CARO'.

The protein profile at the RR stage is not reported for 'CARO' because when their fruits were to be harvested, a disease affected these plants. At the RR stage both cultivated genotypes and wild accession showed 92% polymorphic bands; it was 94.6% in the RILs. Again, a 97 kDa band was detected in all genotypes except for L18 while a 56 kDa band was observed in all genotypes with the exception of 'NOR' and L6. Similarly, a 30 kDa band was present in all genotypes excepting 'NOR' and LA1320. Finally, a 31 kDa band was not observed in two RILs, L6 and L5, and LA1385. Another interesting fact was that polymorphism was detected among different ripening stages for each genotype. LA1246 showed a 39 kDa band that was absent at the MG stage but present in the RR stage while monomorphism was detected for all other bands. For the remaining genotypes, the percentage of polymorphism between ripening stages varied between 29.7% in L18 and 10.8% in L1. **Fig. 2** shows the protein profiles for all genotypes at both ripening stages.

Fig. 3 shows a dendrogram based on the protein profiles of all the genotypes at the MG stage. The cophenetic correlation was 0.46 and the Jaccard distance among genotypes varied between 0.32 and 1.09. Two groups were firstly defined, one including most of the RILs, the accessions LA722, LA1385 and LA1246, and cv. 'UCO'. The second group included the remaining cultivated genotypes ('NOR', 'Caimanta' and 'CARO'), L18 and the accessions LA2181, LA1320 and LA1673.

Fig. 4 shows a dendrogram built with the protein profiles at the RR stage. The cophenetic correlation was 0.52 and the Jaccard distances varied between 0.11 and 1.33. In a first level of clustering, one group was formed by 'UCO' genotype, the accessions LA2181 and LA722 and the RILs L9 and L18, whereas another group was formed by LA1673,

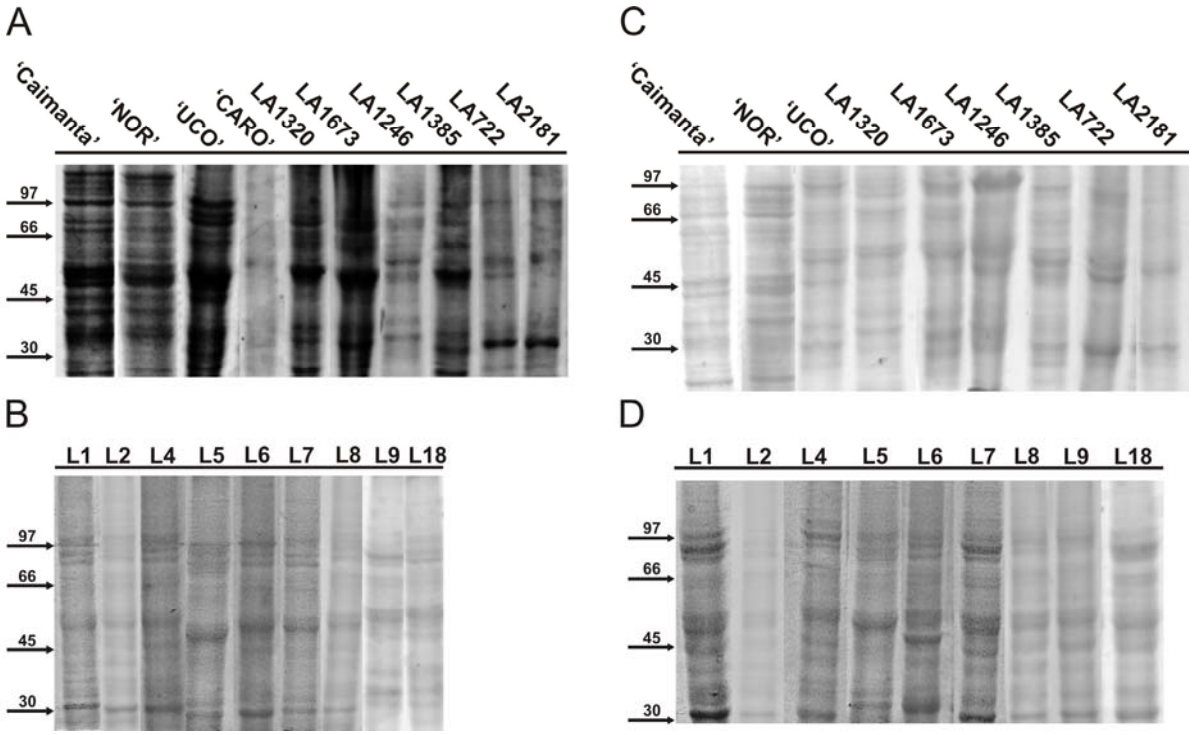

Fig. 2 Protein profiles for all genotypes assayed at the Mature Green (MG) and Red Ripe (RR) stages. (**A, B**) Genotypes at MG, (**C, D**) Genotypes at RR. Arrows indicate molecular weight markers (kDa).

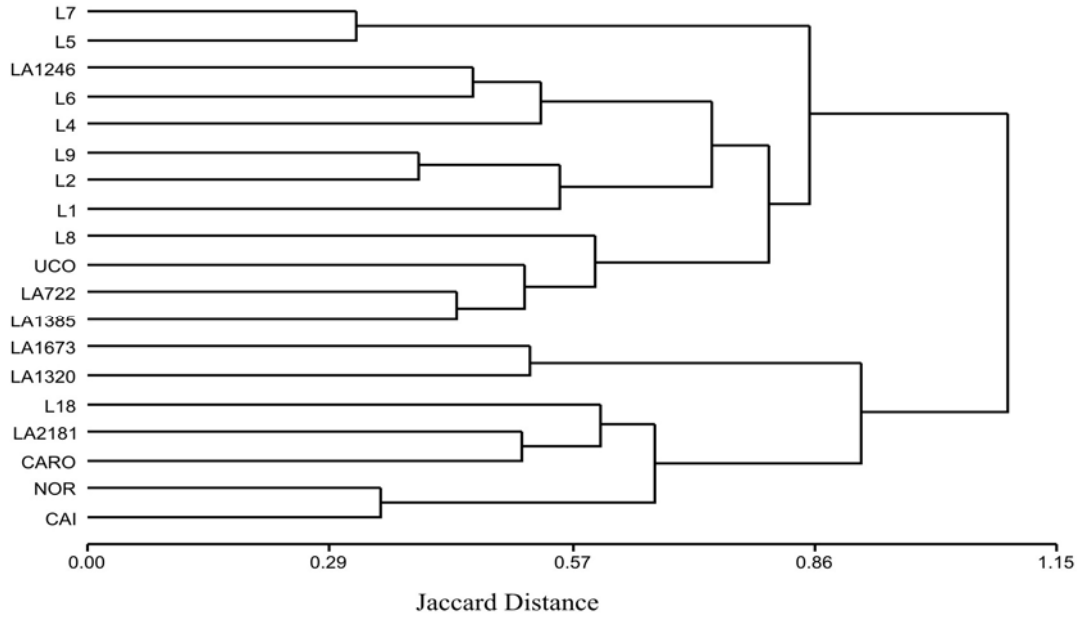

Fig. 3 Dendrogram of protein profiles in all genotypes at the Mature Green (MG) stage according to Ward's method.

LA1320 and LA1385 and, LA1246, the cultivated genotypes 'Caimanta', 'NOR' and the remaining RILs. At a second level, a subgroup was formed by wild accessions LA2181, LA722, and the other was integrated by 'UCO' and lines L9 and L18.

DISCUSSION

This experiment showed significant differences among these sets of genotypes for most of the evaluated traits. Regarding fruit shape only 'CARO' had elongated fruits and only flattened fruits were observed in 'UCO'. The remaining genotypes had round fruits. As pointed before, pericarp thickness and locule number had highest values in those genotypes with greatest fruits. These findings agree with those of Lippman and Tanksley (2001), who found a positive correlation between locule number and fruit size. Consequently, an increase in the number of locules can provoke a significant effect on fruit size. Besides, fruit color is one of the most important quality traits because consumers consider it as an indicator of organoleptic quality. According to our results, there are two genotypes that have strong red color intensity in the fruits: accession LA1246 of *S. pimpinellifolium* and one of the RILs, L6 (see values in **Table 1**). The values found for 'NOR' genotype (a/b= 0.5; L= 48) agree with the visual observations of the fruits. It is known that fruits of this mutant genotype turn in a uniform way from green to pale pink coloration (Tigchelaar *et al*. 1978). Fruit firmness is nearly related with resistance to commercial transport, and is considered another important component of fruit quality for both industry and fresh consumption tomatoes. Our data showed that wild species had mean values of firmness lower than the cultivated species. In fact, 'Caimanta' had the maximum value (F = 60),

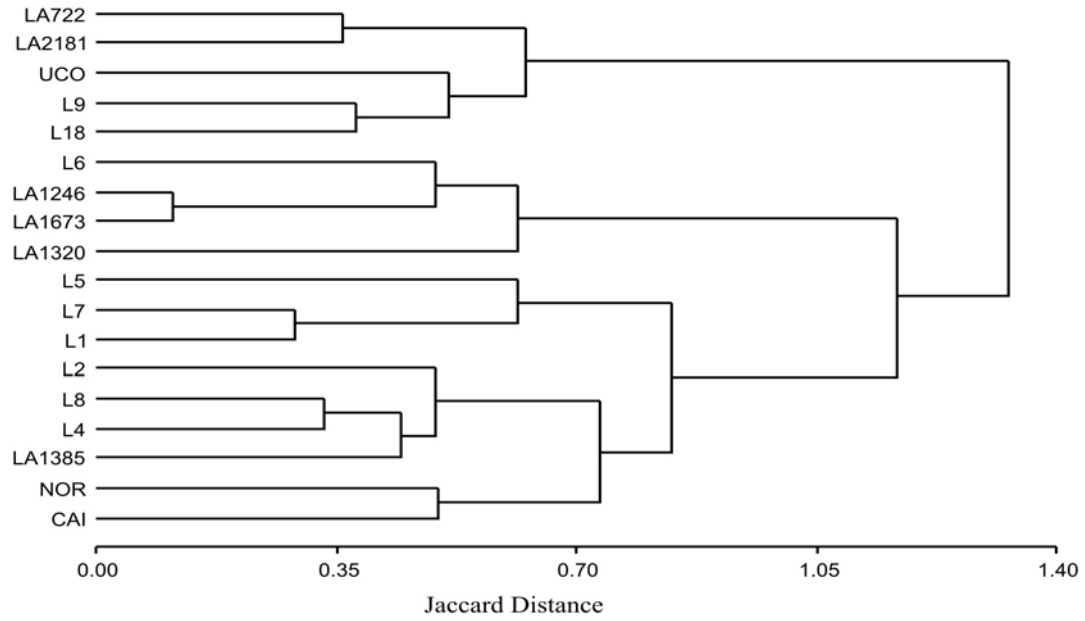

Fig. 4 Dendrogram of protein profile of all genotypes at the Red Ripe (RR) stage according to Ward's method.

whereas the LA2181 accession of *S. pimpinellifolium* showed the lowest (F = 43.5). However, RILs had intermediate values for this trait. With respect to fruit acidity, Stevens (1986) pointed out that higher acidity in fruits does not necessarily imply higher titrable acidity; the lower values of this trait found in cultivars such as 'CARO' and the wild species *S. lycopersicum* var. *cerasiforme* accession LA1673 support this previous claim. The RILs showed pH values near 5 and titrable acidity values that varied between 0.2 and 0.4.

A long fruit shelf life is also a trait highly appreciated in the fresh market. 'NOR' had, as expected, the maximum mean value with a shelf life of 44 days (see **Table 1**). This genotype has a mutant gene located in chromosome 10, in a homozygous state, which alters ethylene production (Tigchelaar *et al*. 1978). It is interesting to remark that wild species showed values higher than the cultivated ones, which is consistent with the results obtained by Zorzoli *et al*. (1998), Pratta *et al*. (2000) and Rodríguez *et al*. (2006a), who demonstrated that wild genotypes of *S. pimpinellifolium* and *S. lycopersicum* var. *cerasiforme* carry genes that prolong fruit shelf life. With respect to fruit weight, it was observed that only the cultivated genotypes had the fruit weight needed to fresh market, which is 100-150 g (Ferratto, pers. comm.). The highest average value was 126 g for 'UCO' and the lowest was 0.7 g for LA2181. For this trait, RILs had values nearer to the wild parent LA722 (**Table 1**). Tomato flavor for fresh consumption is determined principally by the sugar and acid levels, so that increasing the levels of these traits would also increase the flavor (Krumbein *et al.* 2004). The higher values found for soluble solid content in the wild species agree with the results of other authors (Georgelis *et al*. 2006), who tested PI 270248 of *S. lycopersicum* var. *cerasiforme*, 7833-1-1-1 of *S. lycopersicum* and their segregant generations. Among parents, the highest value was 5.00 (PI 270248), varying the F_2 among 2.00 and 5.50. The lowest values for soluble solid content were found in the cultivated species. These results show the importance of fruit quality traits to classify different genotypes.

An important degree of polymorphism was detected by pericarp protein profiles at each ripening stage. Pratta *et al*. (2001) found similar results when they analyzed the protein profiles among tomato genotypes at MG and RR stages. On the other hand, polymorphism between the ripening stages for the same genotype was found. Regarding to this, Carbone *et al*. (2005) found differential expression of genes encoding enzymes of the carotenoid biosynthesis pathway, primary metabolism, photosynthesis and cell wall metabolism at different ripening stages. Faurobert *et al*. (2007) reported that the expression of proteins related to stress and senescence increases during the ripening process in tomato fruits, and most of the proteins related to C-compounds and carbohydrate metabolism or oxidative processes are up-regulated during fruit development, showing an increase in protein during development and maximum abundance in mature fruit.

Miller and Tanksley (1990) estimated that at least 5% of the available genetic variation exists in tomato cultivars and that the remainder is found in wild species of the genus. In our assay, the highest level of polymorphism was found in the MG stage in wild species and RILs while the cultivated species had the lowest level. According to Nesbitt and Tanksley (2002), the species *S. lycopersicum* var. *cerasiforme* is a mixture between cultivated and wild species. This pattern can also be seen in the dendrogram for the MG stage in which two accessions of *S. lycopersicum* var. *cerasiforme* were classified with cultivated genotypes. The taxonomic classification of tomato species has been established based on morphology, cytogenetic and viable crossing capacity (Rick 1973). In our study we used protein profiles at different stages to classify this set of genotypes. This approach allowed grouping at the MG stage of some cultivated genotypes with some wild species (**Fig. 3**). The level of polymorphism among genotypes was higher at the RR stage. We found two monomorphic bands (66 and 30 kDa) in the RILs which were also found by Rodríguez *et al*. (2008) associated with long shelf life when these authors analyzed the F_2 generation from a cross between 'Caimanta' cultivar and accession LA1385. Another interesting fact was that the 97 kDa band was detected in all genotypes with the exception of L18 line. Cluster analysis at the RR stage showed that there is no agreement with the taxonomic classification (**Fig. 4**). These results coincide with Rocco *et al*.'s (2006) results in which specific protein expressed differentially in different cultivated ecotypes of tomato were found. These authors evaluated 'Ailsa Craig' and 'SM2' of *S. lycopersicum* and found that a number of components with a common quantitative trend during fruit ripening would be essential to this physiological phenomenon, while other specific ecotype-related up- or down-regulated proteins should be subject of further studies.

At the MG stage, all RILs except for L18 grouped with LA722 accession whereas at the RR stage all RILs except for L18 and L9 lines were located with the 'Caimanta' genotype indicating the degree of similarity among RILs

and their parents with regards to the ripening process. Nevertheless, the L18 line had an opposite behaviour to the other RILs probably due to differential genetic recombination of parental genomes in this specific RIL.

CONCLUSION

Morphological and biochemical fruit traits allowed for the characterisation of tomato genetic resources, discriminating among cultivated genotypes, wild species and new genotypes as RILs. This was possible because of the wide genetic variability found among them for the evaluated traits. Alternatively, pericarp protein profiles of fruits at the MG and RR stages allowed genotypes to be clustered in a different way than the morphological and biochemical fruit traits.

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REFERENCES

Aivalakis G, Katinakis P (2008) Biochemistry and molecular physiology of tomato and pepper fruit ripening. In: Passam H (Ed) *The Fruiting Species of the Solanaceae*. *The European Journal of Plant Science and Biotechnology* **2 (Special Issue 1)**, 145-155

Babeli PS, Muzzucato A (2008) The Solanaceae – a review of recent research on genetic resources and advances in the breeding of tomato, pepper and eggplant. In: Passam H (Ed) *The Fruiting Species of the Solanaceae*. *The European Journal of Plant Science and Biotechnology* **2 (Special Issue 1)**, 3-30

Carbone F, Pizzichini D, Giuliano G, Rosati C, Perrotta G (2005) Comparative profiling of tomato fruits and leaves evidences a complex modulation of global transcript profiles. *Plant Science* **94**, 165-175

De Luca S, Creus J, O'Grazi D, Dondini L, Bregoli A, Serafín-Fracassini D (2000) Suber vegetative stages and cells cycle in *Helianthus tuberosus* protein pattern and their modification by spermidine. *Plant Physiology* **156**, 17-25

Faurobert M, Mihr C, Bertin N, Pawlowski T, Negroni L, Sommerer N, Causse M (2007) Major proteome variations associated with cherry tomato pericarp development and ripening. *Plant Physiology* **143**, 1327-1346

Garello G, Barthe P, Bonelli M, Bianco-Trinchant J, Le Page-Degivry MT (2000) Abscisic acid regulated responses of dormant and non-dormant embryos of *H. Annus*: Role of ABA-inducible proteins. *Plant Physiology and Biochemistry* **38**, 473-482

Garg N, Devinder SC, Dhatt AS (2008) Genetics of yield, quality and shelf life characteristics in tomato under normal and late planting conditions. *Euphytica* **159**, 275-288

Georgelis N, Scott JW, Baldwin EA (2006) Inheritance of high sugars from tomato accession PI 270248 and environmental variation between seasons. *Journal of the American Society for Horticultural Science* **131**, 41-45

Giovannoni JJ (2004) Genetic regulation of fruit development and ripening. *The Plant Cell* **16 (Suppl.)**, 170-180

Gur A, Zamir D (2004) Unused natural variation can lift yield barriers in plant breeding. *PLOS Biology* **2**, 1610-1615

Hurkman WJ, Tanaka CK (1986) Phenol extraction followed by methanolic ammonium precipitation – an effective protocol for sample preparation from protein-poor, recalcitrant tissues such as plant. *Plant Physiology* **81**, 802-806

Jaccard P (1901) Étude comparative de la distribution florale dans une portion des Alpes et des Jura. *Bulletin de la Socit Vaudoise ses Sciences Naturelles* **37**, 547-579

Krumbein A, Peters P, Brückner B (2004) Flavour compounds and a quantitative descriptive analysis of tomatoes (*Lycopersicon esculentum* Mill.) of different cultivars in short-term storage. *Postharvest Biology and Technology* **32**, 15-28

Lippman Z, Tanksley SD (2001) Dissecting the genetic pathway to extreme fruit size in tomato using a cross between the small-fruit wild species *Lycopersicon pimpinellifolium* and *L. esculentum* var. Giant Heirloom. *Genetics* **158**, 413-422

Miller JC, Tanksley SD (1990) RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. *Theoretical and Applied Genetics* **80**, 437-448

Nakazato T, Bogonovich M, Moyle LC (2008) Environmental factors predict adaptive phenotypic differentiation within and between two wild Andean tomatoes. *Evolution* **62**, 774-792

Nesbitt C, Tanksley SD (2002) Comparative sequencing in the genus *Lycopersicon*: implications for the evolution of fruit size in the domestication of cultivated tomatoes. *Genetics* **162**, 365-379

Padmavatti G, Siddiq EA, Kole C (2001) Inheritance of protein markers detecting polymorphism among rice genotypes with contrasting hot response to green leafhopper. *Current Science* **80**, 1111-1112

Pratta GR, Zorzoli R, Picardi LA (2000) Interacciones genéticas entre germoplasma silvestre y cultivado de *Lycopersicon* spp. Con efectos sobre la calidad del fruto de tomate. *Plant Genetic Resources Newsletter* **124**, 7-12

Pratta GR, Zorzoli R, Picardi LA, Valle EM, Carrillo N (2001) Characterization of tomato genotypes that differ in their fruit shelf-life by analysis of total pericarp protein patterns at two ripening stages. *Acta Horticulturae* **546**, 483-487

Rick CM (1973) Potential genetic resources in tomato species: clones from observations in native habitats. In: Srb AM (Ed) *Genes, Enzymes and Populations*, Plenum Press, New York, pp 205-269

Rocco M, D'Ambrosio C, Arena S, Faurobert M, Scaloni A, Marra M (2006) Proteomic analysis of tomato fruits from two ecotypes during ripening. *Proteomics* **6**, 3781-3791

Rodríguez GR, Pratta GR, Zorzoli R, Picardi LA (2006a) Recombinant lines obtained from an interspecific cross between *Lycopersicon* species selected by fruit weight and fruit shelf life. *Journal of the American Society for Horticultural Science* **131**, 651-656

Rodríguez GR, Pratta GR, Zorzoli R, Picardi LA (2006b) Evaluación de caracteres de planta y fruto en líneas recombinantes endocriadas de tomate. *Ciencia e Investigación Agraria* **33**, 127-135

Rodríguez GR, Sequin L, Pratta GR, Zorzoli R, Picardi LA (2008) Protein profiling in F_1 and F_2 generations of two tomato genotypes differing in ripening time. *Biologia Plantarum* **52**, 548-552

Schauer N, Semel Y, Roessner U, Gur A, Balbo I, Carrari F, Pleban T, Perez-Melis A, Bruedigam C, Kopka J, Willmitzer L, Zamir D, Fernie AR (2006) Profiling of tomato interspecific introgression lines facilitated the identification of pathway and network quantitative trait loci revealing that plant morphological traits are major determinants of fruit metabolic networks. *Nature Biotechnology* **24**, 447-454

Schuelter AR, Finger FL, Casali VWD, Brommonschenkel SH, Otoni WC (2002) Inheritance and genetic linkage analysis of a firm-ripening tomato mutant. *Plant Breeding* **121**, 338-342

Shapiro SS, Wilk MB (1965) An analysis of variance test for normality (complete samples). *Biometrica* **52**, 591-611

Sokal R, Rohlf F (1969) *Biometry*, San Francisco: HW Freeman, 543 pp

Stevens MA (1986) Inheritance of tomato fruit quality components. *Plant Breeding Reviews* **4**, 273-311

Tigchelaar EC, Mc Glasson WB, Buescher RW (1978) Genetic regulation of tomato fruit ripening. *HortScience* **13**, 508-513

Ward JH (1963) Hierarchical grouping to optimize an objective function. *Journal of the American Statistics Association* **69**, 236-244

Zhang LP, Khan A, Niño-Liu D, Foolad MR (2002) A molecular linkage map of tomato displaying chromosomal locations of resistance gene analogs based on a *Lycopersicon esculentum* × *Lycopersicon hirsutum* cross. *Genome* **45**, 133-146

Zhang Q, Riechers DE (2008) Proteomics: an emerging technology for weed science research. *Weed Science* **56**, 306-313

Zorzoli R, Pratta GR, Picardi LA (1998) Efecto de los mutantes *nor* y *rin* y de genes silvestres sobre características del fruto en *Lycopersicon*. *Mendeliana* **13**, 12-19