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ORIGINAL ARTICLE

Wide-genome QTL mapping of fruit quality traits in a tomato RIL population derived from the wild-relative species *Solanum pimpinellifolium* L.

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Abstract
Key message **QTL and candidate genes associated to fruit quality traits have been identified in a tomato genetic map derived from *Solanum pimpinellifolium* L., providing molecular tools for marker-assisted breeding.**
Abstract The study of genetic, physiological, and molecular pathways involved in fruit development and ripening has considered tomato as the model fleshy-fruited species par excellence. Fruit quality traits regarding organoleptic and nutritional properties are major goals for tomato breeding programs since they largely decide the acceptance of tomato in both fresh and processing markets. Here we report the genetic mapping of single-locus and epistatic quantitative trait loci (QTL) associated to the fruit size and content of sugars, acids, vitamins, and carotenoids from the characterization of a RIL population derived from the wild-relative *Solanum pimpinellifolium* TO-937. A genetic map composed of 353 molecular markers including 13 genes regulating fruit and developmental traits was generated, which spanned 1007 cM with an average distance between markers of 2.8 cM. Genetic analyses indicated that fruit quality traits analyzed in this work exhibited transgressive segregation and that additive and epistatic effects are the major genetic basis of fruit quality traits. Moreover, most mapped QTL showed environment interaction effects. *FrW7.1* fruit size QTL co-localized with QTL involved in soluble solid, vitamin C, and glucose contents, dry weight/fresh weight, and most importantly with the *Sucrose Phosphate Synthase* gene, suggesting that polymorphisms in this gene could influence genetic variation in several fruit quality traits. In addition, *1-deoxy-D-xylulose 5-phosphate synthase* and *Tocopherol cyclase* genes were identified as candidate genes underlying QTL variation in beta-carotene and vitamin C. Together, our results provide useful genetic and molecular information regarding fruit quality and new chances for tomato breeding by implementing marker-assisted selection.

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Introduction

Tomato, *Solanum lycopersicum* L., is one of the most relevant horticultural crops in the world because tomato fruit is a major dietary component in many countries being an important source of sugars, minerals, vitamins, and antioxidant compounds (Raiola et al. 2014). Most of these compounds are soluble solids, which contribute to the flavor and processed product yield, and reduce the energy cost of

dehydration in processing tomato production (Rick 1974). Among the most important nutrients of tomato, L-ascorbic acid or vitamin C is essential for cardiovascular function, immune cell development, and iron utilization (Zou et al. 2006). Other nutrients like carotenoids, apart from their functions in tomato fruit ripening, are antioxidants that play an important role in immune system stimulation and preventing cancer and heart diseases (Heber and Lu 2002). The red carotenoid pigment, lycopene, responsible for the mature tomato fruit color, is an important indicator of fruit quality and the main attribute for food-processing tomato industries (Stevens and Rick 1986). Lycopene is not an essential nutrient but it has reported beneficial effects on human health, such as reducing the risk of suffering certain cancers and metastasis (Meadows 2012). Traditionally, fresh markets and food industry have demanded tomato varieties with improved agronomic characteristics such as increased production and resistances, but currently, higher content of nutraceutical compounds is demanded by consumers and producers.

Wild relative species of tomato have been used as donors of some of these fruit quality traits. However, most of these characters, and other agronomic traits like yield and tolerance to biotic or abiotic stresses, are under regulation of several genes, often collectively referred to as polygene or quantitative trait loci (QTL). Therefore, in order to localize these QTL, several linkage maps have been developed from more than 20 mapping populations derived from interspecific crosses between cultivated tomato and their related wild species such as *S. cheesmaniae*, *S. chmielewskii*, *S. neorickii, S. habrochaites,* and *S. pennelli* (reviewed by Foolad 2007). In these maps, different QTL have been identified for one or a few of the main important traits in tomato breeding and even in a small number of cases the gene responsible for the main-effect QTL has been cloned from its map position, i.e., *fw2.2* (Frary et al. 2000). Despite this successful case, phylogenetic distance between most of these wild species and the cultivated one makes unfeasible or at least tedious and inefficient the simultaneous introgression of several traits/QTL into the breeding programs.

Compared to other wild relatives, *S. pimpinellifolium* L. has many desirable traits and significantly fewer unwanted characteristics. Thus, several genetic linkage maps have been developed based on different *S. lycopersicum* × *S. pimpinellifolium* crosses (Grandillo and Tanksley 1996; Chen and Foolad 1999; Lippman and Tanksley 2001; Doganlar et al. 2002; Sharma et al. 2008; Ashrafi et al. 2009). However, most of these maps were developed based on non-permanent populations and all of them contain a high proportion of dominant markers. This, together with the fact that the wild accessions used as parental line did not show enough desirable agronomical traits, reduces the utility of these maps for tomato breeding purposes. Selection of a more appropriated parental donor and stable segregating populations should permit better resolution and usefulness of oncoming genetic maps.

Recently, a new *S. pimpinellifolium* accession named TO-937, which has many interesting horticultural and agronomic characteristics, has been described (Fernandez-Muñoz et al. 2000) and a permanent recombinant inbred lines (RIL) population from a cross between TO-937, and the well-characterized tomato cultivar Moneymaker was developed (Alba et al. 2009). A RIL population has many advantages over other populations that are used for genetic mapping and QTL analysis because each line has a fixed genotype, and the whole population can be distributed and replicated for use in experiments in different laboratories and environments (Ashrafi et al. 2009). This feature is crucial to quantify the effect of genotype × environment (GxE) interaction within a QTL analysis of agronomic traits, especially for fruit quality characters. Additionally, a RIL population is more efficient than the F_2 population from the same parents because fewer individuals are needed to detect linkage of the same magnitude between markers and QTL (Burr and Burr 1991).

In this study, a genetic linkage map based on the RIL population from the TO-937 × Moneymaker cross has been generated. This new map includes a high number of markers (105 SSRs, 233 SNPs, and 15 InDels), all of them codominant, and spans a higher distance than other tomato genetic maps, with an average distance between markers lower than any other interspecific linkage maps generated from previous *S. lycopersicum* × *S. pimpinellifolium* crosses. The mapping population includes 169 RIL that were evaluated for fruit quality traits during two growing seasons. Statistically significant QTL were mapped for characters related to yield as well as soluble solids content, sugars, acidity, organic acids, carotenoids, and vitamin C content. The ultimate goal of this work is the identification of genes involved in the inheritance of all these characters. Therefore, the map reported here contains a high number of candidate genes and QTL, and the latter being co-localized with genes controlling agronomic traits analyzed in this work. We discussed the usefulness of the QTL identified in future tomato breeding programs, as well as novel epistatic relationships between QTL and the QTL × Environment interaction (QTL × E) effects.

Materials and methods

Plant material

A plant of *S. lycopersicum* cv. Moneymaker was crossed to a plant of *S. pimpinellifolium* accession TO-937

(Fernandez-Muñoz et al. 2000) to produce F_1 seeds. Selfing of a single F_1 plant generated an F_2 segregating population. A more advanced segregating plant population composed of 169 recombinant inbred lines (RIL) was generated by single seed descendent (SSD) until the F_8 generation was reached. Four to six biological replicates of each RIL were used for tissue collection, marker genotyping and phenotypic characterization. The agronomic characterization of the RIL was performed during two environmental conditions, a winter–spring and a summer–autumn growing cycles. In all cases biological replicates of each RIL, the two parental lines and the F_1 were grown in a plastic greenhouse under standard commercial growing conditions previously described (Alba et al. 2009). Fruits to be analyzed were collected at red stage and weighted. For each growing cycle, at least twelve fruits per RIL were frozen in liquid nitrogen and kept at −80 ºC until homogenization using a laboratory grinder (IKA A11 Analytical Mill) with its knife and chamber pre-chilled with liquid nitrogen. Resulting homogenated fruit fine powder was used for metabolite analyses.

Determination of total soluble solids content (TSS), titratable acidity (TA), pH, and dry weight/fresh weight (Dw/Fw)

TSS content (ºBrix) was assessed using an ATAGO PR-100 digital refractometer. For titratable acidity determination, 1 ml of the juice was added to 100 ml water and titrated with NaOH 0.01 N (freshly made from a commercial valorated stock of 0.1 N) using a semi-automatic titrator (Schott TitroLine easy) to a final endpoint of pH 8.1. TA was expressed as meq NaOH/100 ml juice. The pH of the fruits was also determined. About 10 ml of thawed juice were weighted and then dried at 80 °C for 3 days and weighted to calculate the Dw/Fw ratio.

Determination of sugar and organic acids

One g of frozen fruit pericarp powder was placed in 80 % ethanol, and sugars and organic acids were extracted from the tissue by heating it to 70 °C as previously described (Miron and Shaffer 1991). Sugars were analyzed using HPLC on a SugarPak column (Waters), taking as reference the retention time of standard samples as well as their refractive index. The citric acid and malic acid content were determined by HPLC, using anion exchange resin, Spherisorb S5C8 (Waters), and an UV–VIS light detector set at 210 nm wavelength. Chromatographic data were handled using TotalChrom™ Workstation software (PerkinElmer).

Determination of vitamin C

Ascorbic acid extraction was performed as follows: 1 ml of extraction buffer (2 % m-phosphoric acid, 2 mM EDTA) was added to 0.15 mg of pericarp frozen powder. Samples were vortexed until thawing and were kept on ice for 20 min. Then, they were spun down, filtered, and carefully transferred to an HPLC vial on ice. Vitamin C (Ascorbic acid) content in fruit tissue was determined using an HPLC equipment (Jasco) with a reversed-phase column (Gemini® 3 µm C18 Phenomenex, Inc.; Kromasil C18, Scharlau) using ultraviolet detection (254 nm). The HPLC mobile phase was 0.1 M NaH_2PO_4 and 0.2 mM Na_2EDTA, pH 3.1 adjusted with orthophosphoric acid.

Carotenoids

Lycopene, b-carotene, and a-tocopherol were extracted and then analyzed using HPLC (C30 reverse-phase stationary matrix) coupled to a continuous photodiode array detection system using a modification of the method described by Fraser et al. (2000). The changes consisted in the use of hexane–acetone–methanol (2:1:1) for sample extraction, using dichloromethane to dissolve the extracted samples, and increasing the HPLC gradient to 100 % methyl *tert*-butyl ether.

DNA isolation and marker analysis

Leaves from parents and progeny plants were collected, quickly frozen in liquid nitrogen and finally ground using a Retsch MM301 mixer mill shaker at maximum speed. DNA was isolated from 100 mg of powdered leaf tissue using DNA easy Plant Mini Kit. Total DNA concentration was estimated by comparison with DNA standards after electrophoresis in 0.8 % agarose gels in 1XTBE (Tris–borate-EDTA). Sets of SNP (Single Nucleotide Polymorphism), SSR (Simple Sequence Repeat), and InDel (insertion/deletion) markers were obtained from the "Solanaceae Genomics Network" (http://solgenomics.net) and the Tomato Mapping Resource Database (http://tomatomap.net/). Among these markers were the following genes: *GDP mannose epimerase1* (*GME1*), *GDP mannose pyrophosphorylase1* (*GMP1*), *Geranyl pyrophosphate synthase* (*GPS*), *Galactose dehydrogenase* (*GalDH*), *Monodehydroascorbate reductase2* (*MDHAR2*), *Lycopene beta-cyclase* (*CRTL1*), *Geranylgeranyl pyrophosphate synthase2* (*GGPS2*), and *Isopentenyl diphosphate isomerase* (*IPI*). Another set of microsatellite markers was selected from previously published results (Smulders et al. 1997; Areshchenkova and Ganal 1999). Marker analysis of the RIL population *S. lycopersicum* × *S. pimpinellifolium* was carried out according to the standard PCR protocols and one of each primer pair was labeled with an Applied Biosystems fluorescent label. Simple sequence repeats (SSR) and InDel loci were amplified in a 10 µl reaction mixture containing 10 ng of template DNA, 50 ng of each primer, and 100 µM of dNTPs, 1.5 mM of $MgCl_2$, 0.2 units of RedTaq DNA polymerase, and 1 × Taq

buffer supplied with the enzyme (Sigma-Aldrich). PCR reactions were performed in an Eppendorf Mastercycler epgradientS thermocycler. The samples were denatured at 94 °C for 3 min, followed by 30 cycles consisting of 94 °C for 15 s, 55 °C for 30 s, and 72 °C for 1 min. SSR and InDel fragments were resolved using a fluorescent dye (FAM, VIC, NED, or PET), and the PCR products were separated by capillary electrophoresis using a DNA sequencer (ABI PRISM® 3130 XL Genetic Analyser, Applied Biosystems, USA). An internal size marker, GeneScan 500 LIZ (35-500 bp; Applied Biosystems), was added, allowing the multiplexing of differently labeled reactions in the same run. Data regarding selectively amplified DNA fragments were analyzed with GeneMapper® Software 3.7 (Applied Biosystems).

Allelic discrimination of SNP genotyping products by high-resolution melting (HRM) analysis was achieved by detecting the difference in melting temperature between the different PCR fragments amplified by each allele. Genomic DNA (10 ng) was mixed with 5 μl of HotShotMastermix (Taq DNA Polymerase, anti-Taq monoclonal antibodies in 2× Reaction buffer with 400 μM dNTP and Stabiliser), 1 μl LCGreen™ Plus + (Idaho Technology Inc.), 1 μl of 10 μM primers, and completed to 10 μl with MQ water according to manufacturer recommendations (Clent Life Science, UK). The HRM PCRs were performed in FramStar 96-well plates (4titude, UK) using the following conditions: 94 °C, 10 min; 45 cycles of two steps, 94 °C, 30 s, 60 °C, 30 s; and a denaturation step of 30 s at 94 °C and renaturation by cooling to 26 °C. Reactions were analyzed and genotyped using a LightScanner® System (Idaho Technology Inc., USA) following standard protocols (Montgomery et al. 2007) as described previously (Salinas et al. 2013).

Linkage mapping

JoinMap® 4 software (van Ooijen 2006) was used to generate the linkage maps. A logarithm of odds ratio (LOD) of 9.0 was established to consider significant linkage, and genetic map construction was performed using Kosambi mapping function (Kosambi 1944) with the following JoinMap parameters: Rec = 0.3, LOD = 2.0, and Jump = 5.

QTL analysis

Quantitative data for QTL analysis were the trait mean values of the RILs at each growing cycle experiment. QTLNetwork 2.0 software (Yang et al. 2008) was used to identify candidate QTL regions for quality traits, single-locus QTL, epistatic QTL (E-QTL) and their environment interaction effects (QTL × environment, QE; and E-QTL × environment, E-QE) across environments (growing cycles). The mixed-model is based on composite interval mapping method (MCIM). It was carried out for both one-dimensional genome scan in order to detect putative QTL and their environment interactions, and two-dimensional genome scan for epistasis. An experimental-wide significant level of 0.05 was designated for candidate interval selection, putative QTL detection, and QTL effect. Both testing and filtration window sizes were set at 10 cM, with a walk speed of 1 cM. The critical F value to identify a QTL was determined by 1000 permutation test. Epistatic and environment interaction effects were estimated using the MCMC (Markov Chain Monte Carlo) method. QTL with only genetic effects were expressed regardless of the environment. QTL with environment interaction were found to have environmentally dependent expression. The genetic map and the QTL detected were drawn using MapChart 2.2 software (Voorrips 2002). Other statistical analyses of the data were performed with SPSS19 statistical package.

Results

Fruit quality characterization of the *S. lycopersicum* × *S. pimpinellifolium* RIL population

The RIL population was cultivated during two growing cycles and the following fruit quality traits were evaluated: fruit weight, total soluble solids, glucose, fructose, fructose/glucose ratio, dry weight/fresh weight ratio, titratable acidity, pH, citric acid, malic acid, vitamin C, lycopene, and beta-carotene. The mean of the phenotypic values observed for the analyzed traits in the parental lines, F_1 and RIL segregating families are presented in Table 1. All the analyzed traits showed continuous variation and transgressive segregation in the RIL population typical of quantitative traits as it is shown for the vitamin C (VitC) content (Fig. 1; see Supplemental Fig. 1 for the rest of analyzed traits). Whereas some of the RIL showed slightly lower VitC content (average 18.01 mg/100 g FW) than the cultivated parent Moneymaker (18.55 mg/100 g FW), other showed higher VitC content (74.01 mg/100 g FW) than the wild parental line (44.47 mg/100 g FW), being the mean VitC content of the RIL more than double that the VitC content of the cultivated parental line (Fig. 1). Several correlations between the traits analyzed during the winter and summer growing seasons were found (Table 2) and are detailed below.

Development of a genetic linkage map based on a RIL population

In order to generate a genetic map of the RIL population, we genotyped the 169 lines with a total of 353 codominant

Table 1 Agronomic characteristic of the parental lines, the F_1 and the range of variation of the analyzed traits in the RIL population

Trait[a]	*S. lycopersicum* cv. Moneymaker[b]	*S. pimpinellifolium* TO-937[b]	F_1^b	RIL[c]
Fruit weight (FrW), g				1.51–58.24
Total soluble solids (TSS), ºBrix	3.45	8.50	5.76	2.63–10.20
Glucose (Glu), µg/mg FW	4.93	7.98	4.58	0.00–29.22
Fructose (Fru), µg/mg FW	5.78	10.47	5.46	0.00–34.99
Fructose/glucose (Fru/Glu)	1.17	1.29	1.19	0.64–3.13
Dry weight/fresh weight (Dw/Fw)	0.05	0.12	0.09	0.06–0.14
Titratable acidity (TA), meqNaOH/100 ml juice	3.78	8.10	5.69	2.19–8.56
pH	4.34	4.45	4.61	4.02–5.31
Citric acid (CA), µg/mg FW	1.08	1.17	1.44	0.00–8.93
Malic acid (MA), µg/mg FW	0.63	0.71	0.44	0.00–9.05
Vitamin C (VitC), mg/100 g FW	18.55	44.47	51.78	18.01–74.01
Lycopene (Lyc), µg/g DW	732.12	812.95	1288.28	229.58–2286.24
Beta-carotene (B-c), µg/g DW	66.29	54.55	94.81	18.77–260.29

[a] Fresh weight (FW) or dry weight (DW)

[b] Mean values of the growing cycles

[c] Range of variation observed in the RIL population

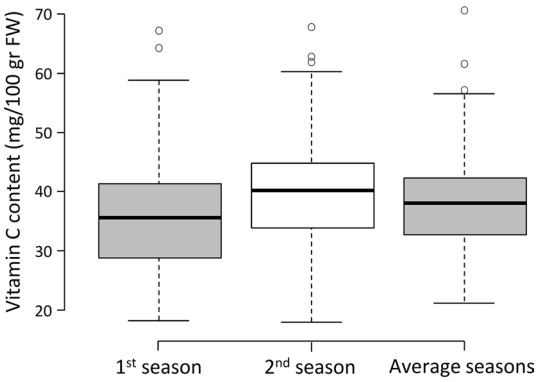

Fig. 1 Vitamin C content in the fruits of the RIL population. *Center lines* show the average values, whereas *box limits* indicate the 25th and 75th percentiles as determined by R software. *Whiskers* extend 1.5 times the interquartile range from the 25th and 75th percentiles and outliers are represented by *dots*. For this trait, the sample size was 163 and 169 for the *first* (winter–spring) and *second* (summer–autumn) growing seasons, respectively

markers, which include 105 SSRs, 233 SNPs, and 15 InDels. To increase our understanding of the genetic basis of fruit quality traits, we also identified SNPs in the sequence of candidate genes like those involved in the synthesis of vitamin C such as *GME1*, *GMP1*, *GalDH,* and *MDHAR2* (Stevens et al. 2007), and use this information to map them. The same approach was used to map genes involved in lycopene biosynthesis such as *GPS*, *GGPS2*, *CRTL1,* and *IPI.* We also mapped the *1-deoxy-D-xylulose 5-phosphate synthase* (*DXS*) gene because it appeared to limit carotenoid biosynthesis during tomato fruit ripening (Lois et al. 2000). Other genes controlling characters of agronomic interest, such as *Fw2.2* (Frary et al. 2000) and *Self Pruning* (Pnueli et al. 1998), were also genotyped and mapped in the population. The genetic map we obtained with these data covered a genetic length of 1007 cM with an average distance between markers of 2.8 cM (Fig. 2), these values being similar to other genetic maps based on populations from *S. lycopersicum* × *S. pimpinellifolium* crosses (Sharma et al. 2008; Ashrafi et al. 2009). The resulting genetic linkage map consisted of 12 linkage groups, and on average, 29 markers were evenly distributed in each chromosome, providing a uniform coverage of the genome. Chromosome length varied from 65 cM (chromosome 8) to 101 cM (chromosome 11), with an average length of 84 cM. Skewed segregation was observed on all chromosomes and, in general, was in favor of both alleles without distinction. This phenomenon has been repeatedly reported in the *S. lycopersicum* × *S. pimpinellifolium* genetic maps previously published (Villalta et al. 2005; Sharma et al. 2008; Ashrafi et al. 2009).

QTL analysis

Fruit weight (FrW)

A negative trait of accession TO-937 is the small size of their fruits, a characteristic of all *S. pimpinellifolium* accessions. Fruit weight (FrW) was found significantly correlated with several fruit quality traits such as titratable acidity (TA) and total solid soluble (TSS) content of the fruits (Table 2). In order to identify QTL associated with FrW, an analysis was carried out on the newly developed

Table 2 Correlations between agronomic traits analyzed in the RIL population

		Frw											
VitC	w	0.117											
VitC	s	−0.003	**VitC**										
TA	w	−0.257**	0.304**										
TA	s	−0.423**	−0.042	**TA**									
pH	w	0.008	0.493**	0.476**									
pH	s	0.006	−0.083	−0.337**	**pH**								
CA	w	−0.052	0.071	0.215**	0.167*								
CA	s	0.053	−0.150	0.100	0.261**	**CA**							
MA	w	−0.021	0.131	0.163*	0.179*	0.795**							
MA	s	0.242**	−0.073	−0.083	0.168*	0.711**	**MA**						
TSS	w	−0.261**	0.415**	0.776**	0.563**	0.281**	0.250**						
TSS	s	−0.415**	0.229**	0.490**	−0.070	−0.024	−0.122	**TSS**					
Glu	w	−0.058	0.173*	0.199**	0.241**	0.082	0.090	0.304**					
Glu	s	−0.292**	0.127	0.183*	−0.181*	−0.418**	−0.462**	0.555**	**Glu**				
Fru	w	−0.048	0.222**	0.210**	0.247**	0.013	0.039	0.337**	0.884**				
Fru	s	−0.306**	0.101	0.154*	−0.145	−0.430**	−0.509**	0.500**	0.970**	**Fru**			
Fru/Glu	w	0.135	0.149	0.123	0.113	0.017	−0.006	0.093	−0.207**	−0.091			
Fru/Glu	S	0.167*	−0.076	−0.122	0.115	0.058	−0.023	−0.215**	−0.193*	−0.009	**Fru/Glu**		
Lyc	w	0.028	0.293**	0.353**	0.470**	−0.038	−0.029	0.321**	0.116	0.128	0.169*		
Lyc	s	−0.021	0.087	−0.096	0.191*	0.149	0.082	−0.070	−0.035	−0.028	−0.099	**Lyc**	
Dw/Fw	w	−0.432**	0.007	0.243**	−0.106	0.044	0.040	0.382**	0.122	0.136	−0.059	−0.028	
Dw/Fw	s	na	na	na	na	na	na	na	na	na	na	na	**Dw/Fw**
B-c	w	0.070	0.296**	0.178*	0.291**	−0.046	−0.064	0.181*	0.234**	0.236**	0.060	0.489**	0.043
B-c	s	0.067	0.302**	−0.003	−0.095	−0.042	0.063	−0.052	−0.096	−0.120	−0.027	0.192*	−0.009

The sign of the correlation coefficient indicates the direction of correlations

w winter–spring growing cycle, *s* summer–autumn growing cycle, *na* data not available or not analyzed in that growing season

* Significant correlation ($P \leq 0.05$), ** Highly significant correlation ($P \leq 0.001$)

S. lycopersicum × *S. pimpinellifolium* genetic map using QTLNetwork 2.0 (Yang et al. 2008). Seven main-effect QTL were detected for FrW, which were located on chromosomes 1, 2, 7, and 11 (Fig. 2; Table 3). All these QTL had positive additive effects indicating that alleles from the cultivated species increased the phenotypic value of this trait (Table 3). The largest effect QTL was *FrW2*, which is flanked by *fw2.2*, a major gene regulator of tomato fruit size (Frary et al. 2000), suggesting that *fw2.2* is responsible for near 15 % of the phenotypic variation in fruit weight observed in the RIL population. Together, the seven main-effects QTL explain more than 49 % of the phenotypic variance observed in FrW (Table 3) although none of them showed additive by environment interaction (AE) effects.

Total soluble solids (TSS), glucose (Glu), fructose (Fru), and fructose/glucose (Fru/Glu)

Total soluble solids content is the most important contributor to tomato processing quality (Grandillo et al. 1999) and is a major determinant of fruit quality for fresh-market consumption (Stevens et al. 1977). As expected, TSS was correlated with glucose (Glu) and fructose (Fru) contents (Table 2). A total of seven additive QTL for TSS in the RIL population were identified which were located on chromosomes 1, 2, 3, 5, 7, and 12. The QTL located on chromosome 2, *TSS2*, explained the highest contribution to the variance of the trait with a value of 14.8 %. Together, the seven QTL could account for 45.3 % of the phenotypic variance and six of them had negative additive values, indicating that positive alleles were provided by *S. pimpinellifolium* (Table 4). The QTL located on chromosome 1, *TSS1*, was also involved in additive interactions (AE) with environment 1 (first growing cycle). This AE interaction

Fig. 2 Genetic linkage map of tomato constructed based on a RIL population of a cross between *S. lycopersicum* cv. Moneymaker and the TO-937 accession of *S. pimpinellifolium*. Twelve linkage groups were constructed using 105 SSRs, 233 SNPs and 15 InDels. The names of the marker and their map position are shown at the *right* and *left* of the chromosomes, respectively. *Boxes* at the *right* of the chromosomes indicate the positions of QTL for the fruit quality traits analyzed

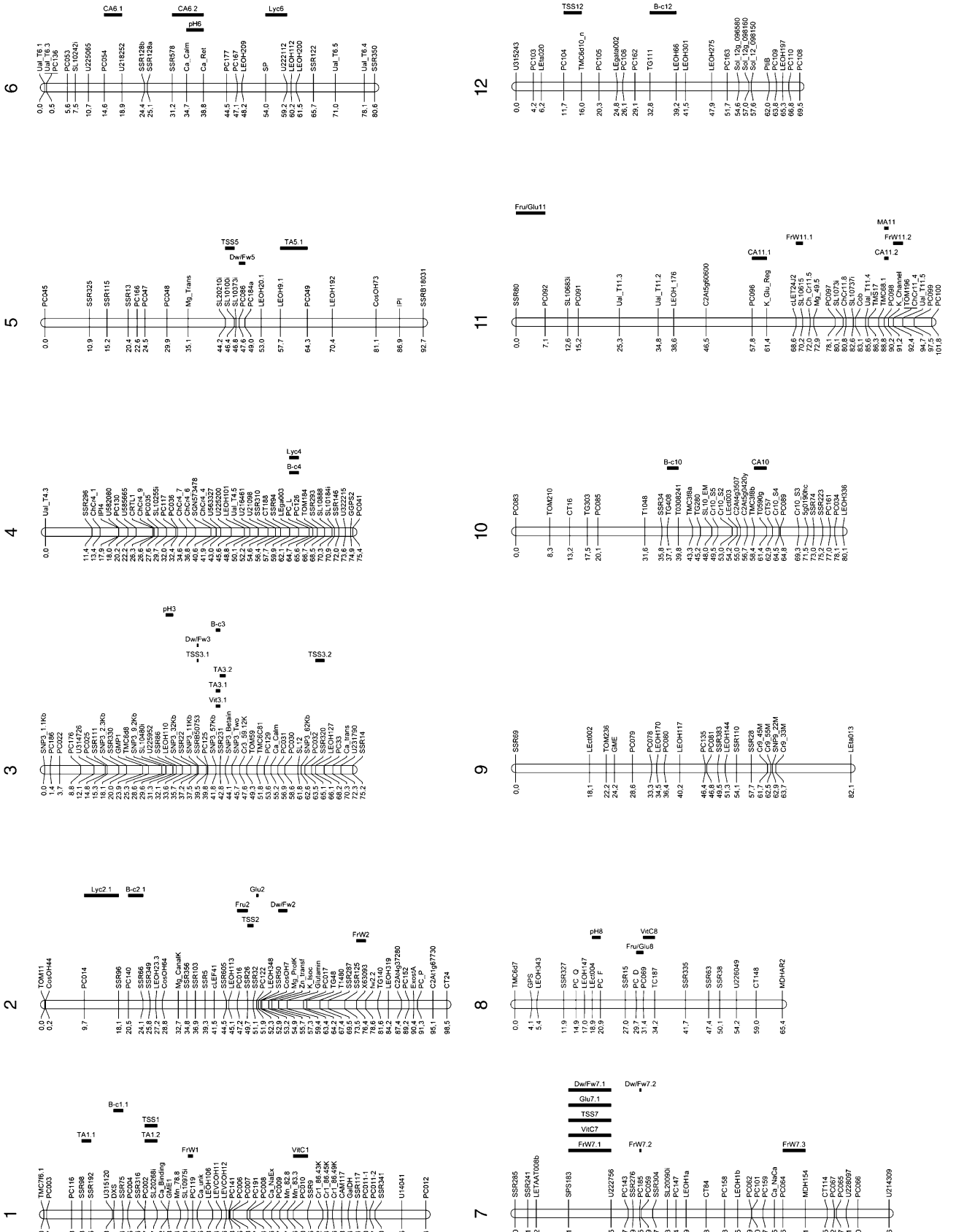
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Table 3 Single-locus QTL and QTL × environment (QE) effects for tomato quality traits identified in a RIL population developed from the cross *S. lycopersicum* × *S. pimpinellifolium* following one dimensional genome scan for multi-environment analysis using QTLNetwork

QTL	Marker interval	LG (pos.)[a]	*F* value[b]	A[c]	h^2(a)[d]	QE AE[e]	h^2(ae)[f]
FrW							
FrW1	SL10975i-PC119	1 (35.3–36.4)	13.7	1.7***	2.3	ns	
FrW2	X63093-**fw2.2**	2 (76.4–78.6)	28.8	6.0***	14.2	ns	
FrW7.1	**SPS183**-U222756	7 (13.1–23.5)	9.3	1.0**	7.1	ns	
FrW7.2	PC185-PC059	7 (30.5–30.9)	14.7	1.3***	7.4	ns	
FrW7.3	PC064-**MDH154**	7 (65.6–71.1)	11.4	2.1***	4.6	ns	
FrW11.1	cLET24J2-SL10615	11 (68.6–70.2)	9.9	3.5***	7.5	ns	
FrW11.2	ChCr11_4-UalT11.5	11 (92.4–94.7)	11.1	4.3***	6.0	ns	
Total soluble solids (TSS)							
TSS1	PC002-SL20268i	1 (24.7–27.7)	8.5	−0.2***	4.8	−0.15* AE1	0.9
TSS2	SSR26-SSR32	2 (49.7–51.1)	20.1	−0.7***	14.8	ns	
TSS3.1	SSR22-SNP3_11 Kb	3 (37.2–37.5)	8.9	−0.4***	9.2	ns	
TSS3.2	LEOH127-PC33	3 (66.1–68.2)	9.8	0.3***	2.2	ns	
TSS5	SL20210i-SL10100i	5 (44.2–46.4)	7.3	−0.2***	2.2	ns	
TSS7	**SPS183-**U222756	7 (13.1–23.5)	12.1	−0.3***	5.4	ns	
TSS12	PC104-TMC6d10_n	12 (11.7–16.0)	7.2	−0.2***	6.7	ns	
Glucose (Glu)							
Glu2	PC122-LEOH348	2 (51.9–52.3)	9.3	−0.8***	4.9	ns	
Glu7.1	**SPS183-**U222756	7 (13.1–23.5)	8.8	−0.6***	2.8	ns	
Fructose (Fru)							
Fru2	PC016-SSR26	2 (47.2–49.7)	8.9	−0.9***	6.4	ns	
Fructose/glucose (Fru/Glu)							
Fru/Glu8	PC_D-PC069	8 (29.7–31.4)	7.0	−0.2**	1.3	−0.3*** AE1	3.1
Fru/Glu11	SSR80-PC092	11 (0.0–7.1)	6.1	0.2**	1.2	0.2** AE1	2.6
Dry weight/fresh weight (Dw/Fw)							
Dw/Fw2	K_Isoc-Glutamin	2 (57.3–59.4)	20.8	−0.0096***	11.5	ns	
Dw/Fw3	SSR22-SNP3_11 Kb	3 (37.2–37.5)	17.3	−0.0063***	9.4	ns	
Dw/Fw5	PC086-PC184a	5 (47.6–49.0)	17.8	−0.0041***	7.5	ns	
Dw/Fw7.1	**SPS183-**U222756	7 (13.1–23.5)	18.3	−0.0034**	7.7	ns	
Dw/Fw7.2	PC185-PC059	7 (30.5–30.9)	26.0	−0.0040***	9.4	ns	
Titriable acidity (TA)							
TA1.1	SSR98-SSR192	1 (9.3–11.5)	9.7	0.3**	3.0	ns	
TA1.2	PC002-SL20268i	1 (24.7–27.7)	10.0	−0.3**	3.6	ns	
TA3.1	SNP3_57 Kb-SSR231	3 (41.8–42.8)	29.0	−0.4**	12.2	ns	
TA3.2	SSR231-SNP3_Betain	3 (42.8–44.1)	20.7	−0.7**	8.0	ns	
TA5.1	LEOH-9.1-PC049	5 (57.7–64.3)	7.5	−0.5**	2.2	ns	
pH							
pH3	SL10480i-U225952	3 (29.6–31.3)	9.3	0.03***	2.3	ns	
pH6	**CaCalm-**CaRet	6 (34.7–38.8)	7.0	−0.02**	1.1	−0.04** AE1	2.1
						0.008*** AE2	2.0
pH8	LEct004-PC_F	8 (18.9–20.9)	14.7	−0.05***	7.9	ns	
Citric acid (CA)							
CA6.1	PC054-U218252	6 (14.6–18.9)	7.2	−0.30*	1.7	−0.20*** AE1	2.0
						0.20*** AE2	2.0
CA6.2	SSR578-Ca_Calm	6 (31.2–34.7)	8.1	−0.20***	4.7	ns	
CA10	TMC3f8b-T0590 g	10 (58.4–61.4)	8.8	−0.20***	5.0	ns	
CA11.1	PC096-K_GluReg	11 (57.8–61.4)	9.3	0.20***	6.0	ns	
CA11.2	PC098-K_Channel	11(90.2–91.2)	11.1	0.67***	6.2	ns	

Table 3 continued

QTL	Marker interval	LG (pos.)[a]	*F* value[b]	A[c]	h^2(a)[d]	QE AE[e]	h^2(ae)[f]
Malic acid (MA)							
MA11	PC098-K_Channel	11 (90.2–91.2)	11.6	0.4***	5.6	ns	
Vitamin C (VitC)							
VitC1	PC010-SSR9	1 (61.0–64.5)	7.4	−5.9***	12.1	ns	
VitC3.1	SNP3_57 Kb-SSR231	3 (41.8–42.8)	11.8	4.2***	5.4	ns	
VitC7	**SPS183**-U222756	7 (13.1–23.5)	11.0	−2.8***	7.6	ns	
VitC8	PC069-**TC187**	8 (31.4–34.2)	7.9	−4.0***	17.0	ns	
Lycopene (Lyc)							
Lyc2.1	PC014-SSR96	2 (9.7–18.1)	8.3	−46.6***	3.4	ns	
Lyc4	SSR94-LEga003	4 (59.9–62.1)	10.5	−57.0***	7.7	ns	
Lyc6	SP-U222112	6 (54.0–59.2)	7.1	−97.7***	5.5	ns	
Beta-carotene (B-c)							
B-c1.1	**DXS**-SSR75	1 (17.1–19.4)	13.4	−9.9***	3.5	−6.5** AE1	0.8
						6.5** AE2	0.8
B-c2.1	PC140-SSR66	2 (20.5–24.1)	9.8	−7.0***	4.0	ns	
B-c3	PC125-SNP3_57 Kb	3 (39.8–41.8)	10.1	−10.4***	4.8	ns	
B-c4	SSR94-LEga003	4 (59.9–62.1)	23.3	22.1***	8.6	12.8*** AE1	1.8
						−12.4*** AE2	1.7
B-c10	TG408-T0308241	10 (37.1–39.8)	10.2	−10.2***	2.9	ns	
B-c12	TG111-LEOH66	12 (32.8–39.2)	13.7	12.2***	6.3	4.4* AE1	0.5
						−5.3* AE2	0.8

ns no significant effects on the four environments evaluated

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. Only significant effects are listed

[a] Chromosome and the estimated confidence interval of QTL position in brackets (in Kosambi cM). Markers corresponding to genes are indicated in bold

[b] *F* values of significance of each QTL. Threshold *F* values were 9.10, 7.0, 6.9, 6.8, 6.7, 6.6, 6.9, 7.1, 7.0, 5.6, 14.8, 6.9 and 7.5 for FW, VitC, TA, pH, CA, MA, TSS, Glu, Fru, Fru/Glu, Dw/Fw, Lyc and B-c, respectively

[c] Estimated additive effect. Positive values indicate that alleles from *S. lycopersicum* increase the trait value, and negative values indicate that the increase in the trait is due to the presence of the alleles from *S. pimpinellifolium*

[d] Percentage of the phenotypic variation explained by additive effects

[e] Predicted additive by environment interaction effect. AE1, AE2 additive by environment interaction effect associated with first and second growing cycles environments, respectively. The sign values indicate the same as shown in A[c]

[f] Percentage of the phenotypic variation explained by additive by environment interaction effect

explained an additional 0.9 % of the phenotypic variance of TSS. When the concentration of the main sugars was determined, we found two QTL, *Glu2* and *Glu7.1* that explained 4.9 % and 2.8 % of the phenotypic variance for fruit Glu content, respectively. Interestingly, one of these QTL was located near to *TSS2* on chromosome 2, whereas the other QTL was found on the same chromosomal region as *TSS7* (Fig. 2). Both QTL had negative additive value effects on Glu content indicating that alleles from *S. pimpinellifolium* contribute to increase phenotypic value of this trait. No additive by environment interaction (AE) effects were identified for main-effect QTL *Glu2* and *Glu7.1*. However, we found one epistatic interaction between two QTL located on chromosomes 1 and 7 explaining 2.7 % of the phenotypic variance of fruit Glucose content. This epistatic interaction did not show additive interaction with the environments.

Concerning Fru content of the fruits, only one main-effect QTL, *Fru2*, was detected located on chromosome 2 in a position very close to *TSS2* and *Glu2*. This QTL explained 6.4 % of the phenotypic variance and had an additive value of -0.9, which indicate that positive alleles for this trait were provided by *S. pimpinellifolium*. *Fru2* showed neither any interaction with the environment (AE) nor any epistatic interaction between QTL implicated in the inheritance of fruit Fru content.

Given that Fru is sweeter than Glu, the ratio Fru/Glu is an important trait in tomato breeding because, with the same amount of sugars, sweeter fruits can be obtained by increasing this ratio. Although parental lines showed quite

Table 4 QTL interactions involving epistatic (E-QTL) and E-QTL × environment (E-QE) effects for quality traits detected in a RIL population developed from the cross *S. lycopersicum* × *S. pimpinellifolium* following two-dimensional genome scan for multi-environment analysis using QTLNetwork

E-QTLi[a]	Marker interval	LG (pos.)[b]	E-QTLj[a]	Marker interval	LG (pos.)	*F* value[c]	AA[d]	h^2(aa)[e]	E-QE AAE[f]	h^2(aae)[g]
Glucose (Glu)										
Glu1	CaBinding-**GME1**	1 (30.2–30.3)	*Glu7.2*	PC059-SSR304	7 (30.9–33.7)	6.5	0.6***	2.7	ns	
Frucose (Fru)										
Fru3	SNP3_32 Kb-SSR22	3 (35.7–37.2)	*Fru12*	LEta020-PC104	12 (6.2–11.7)	7.8	−0.9***	4.2	ns	
Titriable acidity (TA)										
TA1.3	PC004-SSR316	1 (20.5–24.6)	*TA3.3*	U314726-PC025	3 (12.1–14.8)	8.7	−0.2***	2.9	ns	
TA4.1	SSR296-ChCr4_1	4 (11.4–13.4)	*TA7.1*	LEOH_1.1-CT84	7 (41.9–46.8)	9.7	0.2***	3.0	ns	
TA4.2	U573478-ChCr4_4	4 (40.6–41.9)	*TA11*	Ual_T11.5-PC099	11 (94.7–97.5)	7.0	1.3***	3.4	ns	
TA5.2	**IPI**-SSRB18031	5 (86.9–92.7)	*TA9*	LEct002-TOM236	9 (18.1–22.2)	7.0	−0.3***	5.8	ns	
TA7.2	SSR304-SL20090i	7 (33.7–37.3)	*TA10*	TG303-PC085	10 (17.5–20.1)	7.4	−0.2***	4.3	ns	
pH										
pH2	SSR96-PC140	2 (18.1–20.5)	*pH6*	Ca_Calm-Ca_Ret	6 (34.7–38.8)	8.9	−0.02**	1.0	ns	
Citric acid (CA)										
CA2	CosOH64-Mg_CanalK	2 (28.8–32.7)	*CA11.3*	K_GluR-cLET24J2	11 (61.4–68.6)	10.8	−0.42**	3.6	ns	
Malic acid (MA)										
MA2.1	SSR50-CosOH7	2 (52.9–53.2)	*MA2.2*	Zntransf-K_Isoc	2 (55.7–57.3)	8.3	−0.1***	4.3	ns	
Vitamin C (VitC)										
VitC3.2	Ca_Calm-PC031	3 (55.2–56.9)	*VitC5*	LEOH192-CosOH73	5 (70.4–81.1)	9.4	10.5***	5.5	ns	
Lycopene (Lyc)										
Lyc2.2	LEOH348-SSR50	2 (52.3–52.9)	*Lyc3.1*	SSR22–SNP3_11 Kb	3 (37.2–37.5)	11.2	−122.7***	4.1	−93.3* AE1	1.4
								1.6	98.7* AE2	1.6
Lyc3.2	U314726-PC025	3 (12.1–14.8)	*Lyc7*	PC064-**MDH154**	7 (65.6–71.1)	10.7	−61.7***	3.1	ns	
Beta-carotene (B-c)										
B-c1.2	U14041-PC012	1 (87.5–93.4)	*B-c2.2*	SSR125-X63093	2 (73.5–76.4)	12.4	38.3***	3.3	ns	
B-c2.3	LEOH319-C2At4g37280	2 (84.2–87.4)	*B-c5*	LEOH91-PC049	5 (57.7–64.3)	7.1	6.5***	2.1	ns	

ns no significant effects on the four environments evaluated

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. Only significant effects are listed

[a] E-QTLi and E-QTLj are the two QTLs involved in epistatic interaction

[b] Chromosome location and the estimated confidence interval of QTL position in brackets (in KosambicM). Marker corresponding to genes are indicate in bold

[c] *F* values of significance of each epistatic interaction. Threshold *F* values were 5.5, 6.6, 6.9, 6.8, 4.8, 3.3, 5.4,5.4, 6.8 and 7.0 for VitC, TA, pH, CA, MA, Glu, Lyc and B-c, respectively

[d] Estimated additive by additive epistatic effect. Positive values indicate that alleles from *S. lycopersicum* have a positive effect on the traits and negative values indicate that positive effect on the traits is due to the presence of the alleles from *S. pimpinellifolium*

[e] Percentage of the phenotypic variation explained by additive by additive epistatic effects

[f] Predicted additive by additive epistatic effect by environment interaction effect as in c. AAE1 and AAE2: epistasis associated with the first and second growing cycles environment, respectively. The sign values indicate are as shown in additive by additive epistatic effect

[g] Percentage of the phenotypic variation explained by additive by additive epistatic effect by environment interaction effect

similar Fru/Glu ratio, the RIL lines showed transgressive segregation for this trait (Table 1), which facilitated the mapping of two QTL located on chromosomes 8 and 11 (Fig. 2). They explained 2.5 % of the total phenotypic variance and both QTL were involved in AE interactions, which in turn explain 5.7 % of phenotypic variance of this trait (Table 3).

Titratable acidity (TA), pH (pH), citric acid (CA), and malic acid (MA)

Fourteen main-effects QTL were identified for acidity-related traits: 5 for titratable acids (TA), 3 for fruit pH (pH), 5 for citric acid content (CA), and 1 for malic acid content (MA). QTL for pH and CA are located on an overlapping region of chromosome 6 and QTL for CA and MA are located in the same region of chromosome 11 suggesting a common genetic base for these two pairs of characters. Regarding TA, the five QTL identified on chromosomes 1, 3, and 5 accounted for 2.2–12.2 % of the phenotypic variance. Wild alleles of four out the five QTL increased the fruit acidity and none showed environment interaction effects (Table 3). On the other hand, five epistatic interactions located on chromosomes 1–3, 4–7, 4–11, 5–9, and 7–10 accounting for 19.4 % of the phenotypic variation of the TA were found. These epistatic interactions did not show any significant interaction with the environment (Table 4).

For pH of the fruit (pH), we found 3 QTL with additive effects that significantly influenced pH content located on chromosomes 3, 6, and 8. The QTL on chromosomes 6 and 8 explained 1.1 and 7.9 % of the phenotypic variance, respectively, and they had negative additive effects on pH; contrarily, the chromosome 3 locus, *pH3*, showed a positive additive effect on the character. These results suggested that although alleles from both parents contributed to the inheritance of fruit pH, alleles of wild parent explain threefold higher percentage of the variance observed for this taste-related trait. The QTL located on chromosome 6, *pH6*, showed an interaction with the two environments of the assays corresponding to the two growing cycles of the RIL. One pair of epistatic QTL located on chromosomes 2 and 6 was identified for pH content of the fruits. The contribution of the epistatic QTL interaction to pH was only 1 %, and they are alleles from the *S. lycopersicum* parental line.

Citric acid (CA) content has a major effect on tomato flavor and consumers' acceptability. When CA content of the fruit was analyzed we found 5 QTL located on chromosomes 6, 10, and 11. The trait-increasing QTL alleles on chromosomes 6 and 10 came from the wild parent *S. pimpinellifolium* and together explained 11.4 % of the phenotypic variance. The *CA6.1* QTL was also involved in AE interactions with the environment, which explained 2 % of the phenotypic variance of CA. Since *CA11.1 and CA11.2* had positive additive values (Table 4), alleles from both parents contributed to the increase CA content in tomato fruits. One epistatic interaction among the QTL implicated in fruits CA content was also found (Table 4).

Malic acid (MA) is used as an additive for processing tomato because it enhances tomato flavor. When MA content was analyzed in the RIL population, we only found one QTL located on chromosome 11 with an additive value of 0.4 and explaining 5.6 % of the phenotypic variance. We also found an epistatic interaction that explained a phenotypic variance of 4.3 % and had an additive value of 0.1 on MA content (Table 4). All these acidity-related traits were correlated among themselves during the two growing seasons that fruits of the RIL population were analyzed, and they also showed significant positive correlations with TSS, Glu, and Fru (Table 2).

Dry weight/fresh weight (Dw/Fw)

The fruit dry weight/fresh weight ratio is a very important trait especially in breeding programs focused on varieties for the processing industry. When the inheritance of this trait was analyzed, a total of five QTL were found, one located on chromosomes 2, 3, and 5 and two QTL on chromosome 7. Together the four QTL explained more than 45.5 % of the phenotypic variance and all alleles derived from *S. pimpinellifolium* contribute positively to this trait. The QTL *Dw/Fw2* had the highest contribution and explained 11.5 % of the phenotypic variation in Dw/Fw of the fruits. *Dw/Fw7.1*, co-localized with *TSS7* suggesting the existence of a similar genetic basis for both characters. None of the QTL for the Dw/Fw ratio showed additive interactions with the environment (AE), and no epistatic interactions among QTL involved in Dw/Fw inheritance were found.

Vitamin C (VitC)

Accession TO-937 of *S. pimpinellifolium* can be considered a donor of high vitamin C (VitC) content because their fruits showed 2.4-fold more VitC content than the fruits of cv. Moneymaker (Table 1). VitC content showed highly significant positive correlations with TSS and B-c content during the two growing cycles analyzed (Table 2). Four main-effect QTL for VitC content of the fruit were located on chromosomes 1, 3, 7, and 8 (Fig. 2; Table 3). Their additive values ranged from −5.9 to 4.2, whereas the phenotypic variance explained by these QTL ranged from 5.4 to 17.0 %. Three QTL (*VitC1*, *VitC7,* and *VitC8*) had negative additive effects values on vitamin C content of the fruit, indicating that wild alleles increase vitamin C content,

while the other QTL had the opposite additive effect. This result demonstrates that alleles from both parents contributed to the increasing of vitamin C content. The total contribution of the four main-effect QTL on vitamin C variation was 42.1 %, and no additive by environment interaction (AE) effects were identified for any of these QTL.

When epistatic interactions involved in VitC were analyzed, a pair of epistatic QTL were identified on chromosomes 3 and 5 (Table 4). This interaction explained a phenotypic variance of 5.5 %. Interaction between QTL *VitC3.2* and *VitC5* showed a positive additive by additive epistatic effect indicating that alleles increasing vitamin C content come from the cultivated genome (Table 4).

Carotenoids: lycopene (Lyc) and beta-carotene (B-c)

Carotenoids are tetraterpenoid pigments essential for animal diets that are synthesized in photosynthetic organisms. We determined the content of the two most common carotenoids in mature tomato fruits, the linear lycopene, and the vitamin A precursor cyclic beta-carotene in fruits of the RIL population. We found three QTL located on chromosome 2, 4, and 6 involved in the inheritance of Lyc content of the fruit (Fig. 2). These QTL, which accounted for 16.1 % of the total phenotypic variance, increased the lycopene content of the fruit and their additive effects indicated that the *S. pimpinellifolium* alleles have positive effect on this trait (Table 3). These main-effect QTL were not involved in any environment interaction. However, two pairs of epistatic QTL interactions that explained a phenotypic variance ranging from 1.6 to 4.1 for lycopene content were identified on chromosomes 2–3 and 3–7. The total contribution of epistatic QTL was 8.8 % and showed significant interaction effects across environments (Table 4).

When beta-carotene content (B-c) was analyzed, a total of six QTL, located on chromosomes 1, 2, 3, 4, 10, and 12 (Table 3) were identified for this trait. These QTL increased beta-carotene and accounted for a phenotypic variance ranging from 2.9 to 8.6 %. The trait-increasing QTL alleles of *B-c1.1*, *Bc-2.1B-c3,* and *B-c10* came from the *S. pimpinellifolium* parent and whereas those of *B-c4* and *B-c10* derived from *S. lycopersicum*. This suggested that the alleles for high B-c content of the fruits were dispersed within the genomes of the two species (Fig. 2). Three QTL were involved in AE interactions, which explained 6.4 % of the phenotypic variance of B-c content of the fruits. One main-effect QTL *B-c1.1* was flanked by the *DXS* gene, suggesting that this gene could be responsible of the B-c content present in the RIL fruits. The QTL identified on chromosome 4 was found located in the same chromosomal region than the lycopene QTL *Lyc4*. Two pairs of epistatic QTL for B-c were resolved and explained a phenotypic variance ranging from 2.1 to 3.3 %. The epistatic interaction *B-c1.1-B-c2.2* had the largest effect, with a 3.1 % of the phenotypic variance. The total contribution of epistatic QTL interactions was 5.4 % of the phenotypic variance.

Discussion

Genetic analyses reported here emphasize the importance of developing and characterizing RIL populations generated from wild species as a source of genetic and phenotypic variability and also its value to map QTL associated to fruit quality traits in a model fleshy-fruited species as tomato. Indeed, the TO-937-derived RIL population used in this study has already facilitated the isolation of new regulatory genes and markers of undoubted agronomic interest (Powell et al. 2012; Salinas et al. 2013). Our results show that all traits analyzed exhibited transgressive segregation and the identification of a set of molecular markers associated to significant fruit quality QTL will provide useful information and molecular tools for tomato breeding. In the following sections, the discussion has been focused on the significance of the QTL characterized in this work.

Genetic analysis and mapping of fruit quality traits

Genetic analyses performed in the RIL population confirm that inheritance of quality components of tomato fruit is complex in nature, and results from a large number of major and minor QTL. In fact, we have identified main-effect QTL as well as other QTL involved in epistatic or environmental interactions. Despite the fact that epistasis is to be expected in traits that are controlled by several genes/QTL in autogamous plants (Holland 2001), few studies of epistatic interactions have been reported in conventional tomato segregating populations so far (Grandillo and Tanksley 1996; Saliba-Colombani et al. 2001). In the current study, epistasis was detected for nine of the thirteen quality traits evaluated and 15 epistatic interactions were identified. Except for the QTL *pH6*, which showed both main and epistatic effects, the remaining QTL detected by the two-loci QTL analysis displayed only epistatic effects. These epistatic QTL might be considered as modifying genes, which have no significant effects alone but might affect its expression by epistatic interactions with other loci. Thereby, not only the main-effect QTL but also the epistatic QTL detected in this work provide genetic information useful for pyramiding several quality traits in tomato and should be considered for a successful application of Marker-Assisted Selection (MAS).

Genetic linkage maps are essential tools for practical applications such as marker-assisted selection (MAS) and the map-based cloning of target genes, for which a correct linear order of loci within linkage groups is essential.

We have developed a novel genetic linkage map based on the RIL population *S. lycopersicum* cv. Moneymaker × *S. pimpinellifolium* accession TO-937. When compared with the several *S. lycopersicum* × *S. pimpinellifolium* linkage maps previously published (Grandillo and Tanksley 1996; Chen and Foolad 1999; Lippman and Tanksley 2001; Doganlar et al. 2002; Villalta et al. 2005; Sharma et al. 2008; Ashrafi et al. 2009), it can be concluded that this map also provided additional usefulness. While most of those maps were constructed based on crosses between the processing tomato cultivar M82 and accessions LA1589 or LA2093 of *S. pimpinellifolium*, the genetic map reported here was generated using cv. Moneymaker and TO-937, which add some novelties to the parental lines used in previous studies. Indeed, cv. Moneymaker is a fresh-market tomato widely used for genetic studies, and TO-937 is a valuable donor of pest resistances and fruit quality traits (Fernandez-Muñoz et al. 2000; Rodríguez-López et al. 2011; Powell et al. 2012; Salinas et al. 2013). Moreover, TO-937 and LA1589 (the first *S. pimpinellifolium* accession that has been sequenced) showed relatively low genetic similarity in a survey using a large set of SNP markers in a sample of *S. pimpinellifolium* accessions (data to be published elsewhere).

In addition, the nature of the segregating population we used to construct the map differs from most of the previously reported similar maps. We used a RIL population, which allowed us to evaluate each genotype by phenotyping several plants in two different environments (growing seasons). And finally, all the markers we mapped are codominant and most of them correspond to genes of interest for the tomato breeding programs, such as those involved in the control of fruit size and the biosynthesis pathways of lycopene or vitamin C, among others. The utility of this map could be increased after the release of the sequence of the tomato genome and the pre-release of the whole-genome shotgun assembly for *S. pimpinellifolium* accession LA1589 (The Tomato Genome Consortium, 2012). More recently, a huge amount of SNP arisen from the resequencing of quite diverse accessions of tomato have been reported (Xu et al. 2013; Aflitos et al. 2014; Lin et al. 2014), and therefore, novel markers could be included in the map. Moreover, some of the RIL here characterized accumulate several beneficial QTL, and hence they can be selected as donor parental lines in tomato breeding initiatives. The identification of novel lines containing several agronomically interesting characters conveys an advantage of the QTL mapping approach used in this work over the association mapping recently performed in a large collection of tomato accessions (Xu et al. 2013; Lin et al. 2014). Despite the increase availability of tomato and wild tomato relatives genomes is facilitating GWAS studies (Xu et al. 2013; Lin et al. 2014), there is still a need for QTL analysis based on biparental populations to capture all untapped natural variability of these species.

Fruit weight, soluble solid, and sugars

TO-937 is derived from a wild accession of *S. pimpinellifolium*, thus it develops small-sized fruits. To avoid linkage drag of this trait, it is important to know where the genetic determinants of fruit size are located. The QTL with the largest effect on fruit weight was *FrW2,* and it was flanked by *fw2.2* in chromosome 2 (Fig. 2), a gene previously described as a major QTL that, depending on the population studied, accounting up to 30 % of the variation in fruit weight and it is considered one of the most important step in tomato domestication (Frary et al. 2000; Nesbitt and Tanksley 2001). This QTL explained more than 14 % of the phenotypic variation observed (Table 3) in the RIL population used in this work.

Other QTL with minor effect on fruit weight in the RIL were located on chromosomes 7 and 11 (Table 3). In previously published works, QTL affecting fruit weight were found located on chromosomes 7 and 11 (Chen et al. 1999). Although this latter map does not share enough common markers with the map here constructed to allow a comparison between them, our results indicate that these chromosomes may contain multiple closely linked QTL for this trait. In addition, we found epistatic relationships between QTL controlling fruit weight indicating that this character could be controlled by a more complex polygenic and epistatic genetic architecture than the one previously described by Chen et al. (1999).

Association mapping approach recently performed in a large collection of cultivated tomato accessions and in wild-related species identified 16 QTL implicated in the inheritance of fruit weight (Xu et al. 2013), some of them being novel QTL while others co-localize with previously mapped QTL. Recent genomic analyses performed in a wide collection of tomato accessions identified 18 loci involved in the evolution of fruit mass, some of them related to domestication and improvement of this trait (Lin et al. 2014). Our approach does not allow us to distinguish QTL involved in these two important steps of the tomato breeding story, but having in mind the origin of the parental lines of the RIL, QTL located on chromosomes 1 and 7 could be associated to domestication, whereas QTL on chromosomes 2 and 11 could be related to the breeding effort performed to obtain the cv. Moneymaker.

High-soluble solids content not only increase processed fruit yield, but also reduce the energy cost of dehydration in processing tomato production (Rick 1974). High-soluble solids content also increases the fruit overall flavor in fresh-market tomato (Stevens and Rick 1986; Khialparast et al. 2013). Given the importance of this trait, it is one of the

most analyzed in tomato and the number of QTL controlling this trait differs notably depending on the mapping population employed, from just one QTL reported after analyzing sub-NILs or NILs (Monforte and Tanksley 2000; Frary et al. 2003), up to even 23 QTL located on most chromosomes were identified in a introgression line population (Eshed and Zamir 1995). Most of these works had been performed using genetic maps mainly based on anonymous RFLP markers. However, markers included the map here described are mainly gene specific sequences, which allowed us to identify a SNP polymorphism (SPS183 marker) in the sequence of the *Sucrose phosphate synthase* (Fig. 2) linked to *FwR7.1* and other QTL. This result suggests that *FwR7.1* QTL could contain a candidate gene influencing fruit quality traits of tomato such as TSS, sugar content, and Dw/Fw ratio.

Fruit acidity

Titratable acidity (TA) and pH are both important criteria for tomato organoleptic quality and during fruit processing since low pH in fruits allows a reduction in processing time (Kader et al. 1977; Baldwin et al. 1991). We found four QTL for fruit titratable acidity which are located in chromosomes 1, 3, and 5. QTL located on chromosome 1 and 3 co-localize to those previously described both in a RIL population from an intraspecific cross (Causse et al. 2004) and in IL population derived from *S. pennelli* (Saliba-Colombani et al. 2001), indicating that they may include major determinants of fruit acidity. In addition, TA and pH traits showed a highly significant correlation between them and also with MA, TSS, Glu, Fru, Lyc, B-c, and A-t. Additionally, TA was correlated with Suc and Dw/Fw and VitC, indicating that breeding for fruit acidity could increase other-related fruit quality traits (Table 2).

Regarding pH of tomato fruit, QTL involved in the control of this trait had been detected on all chromosomes of tomato genome, although with quite different results being obtained depending on the segregating population and the wild parental donor used in each study (reviewed in Foolad 2007). We have found QTL for pH on the same chromosomes than Fulton et al. (2002) did from backcross populations of *S. peruvianum*, *S. hirsutum*, *S. parviflorum,* and *S. pimpinellifolium*. In addition, we found epistatic interactions between one of these QTL (i.e., *pH6*) and a QTL located in chromosome 2 (Table 4), being this the first report about this kind of genetic interaction as involved in the control of pH of tomato fruit.

Tomato fruits accumulate two main types of organic acids during ripening, i.e., citric acid and malic acid. Although malic acid tastes sourer to consumers, citric acid produces most of the sour taste in tomato because it is accumulated at higher levels. In addition citric acid and malic acid contents in the fruit of the RIL population were highly correlated (Fig. 1). We found only one main-effect QTL (Table 3) involved in malic acid content (Table 4) and a pair of QTL with epistatic interaction. These QTL lie in different chromosomal locations to those already described in a segregating population generated from a different donor parent (Causse et al. 2004). On the contrary, citric acid is controlled by 7 QTL, two of them (*CA2* and *CA11.3*) showing epistatic interaction. One of these QTL showed significant interaction with the environment and another QTL is located on chromosome 10 in position similar to one of the QTL controlling citric acid already described (Causse et al. 2004). These results seem to corroborate that both orthologous and species-specific genes could be involved in fruit quality traits.

Tomato antioxidants: vitamin C and carotenoids

Tomato antioxidants include vitamins such as ascorbic acid and tocopherols, phenolic compounds such as flavonoids, and carotenoids such as beta-carotene, a precursor of vitamin A, and mainly lycopene, which is largely responsible for the red color of the fruit (Moco et al. 2006; Borguini and Torres 2009; Kotkov et al. 2009; Vallverdú-Queral et al. 2011). These compounds have been proposed to play important roles inhibiting reactive oxygen species responsible for many diseases (Crozier et al. 2009; Raiola et al. 2014). Lycopene is a carotenoid widely present in tomato and dietary intake of food containing lycopene has been shown to be related to a decreased risk of chronic diseases, such as cancer and cardiovascular disease (Agarwal and Rao 2000). Lycopene content in tomato fruit has been described as a quantitative trait controlled by QTL located on several chromosomes, depending on the wild donor species, and indeed all tomato chromosomes, except to chromosome 9, seem to contain at least one QTL involved in lycopene content (see Foolad 2007 for a review). However, chromosome 4 is the only chromosome that contains QTL controlling lycopene content in several wild species. Interestingly, this QTL seem to be located in *S. peruvianum* and *S. habrochaites* (Yates et al. 2004) in a syntenic position to *Lyc4*, one of the main-effect QTL controlling lycopene content in the RIL population reported in this work. This QTL co-localize with one of the 6 QTL controlling beta-carotene content in the fruit, which could explain the correlation showed by these fruit quality traits. We mapped carotenoid-related genes *GGPS2* and *IPI* (Van der Hoeven et al. 2002), *IPI4*, *CRTL1* (Cunningham et al. 1996), *GGPS* (Ament et al. 2006), and *DXS* (Lois et al. 2000). Only map position of *DXS* overlapped with the QTL for beta-carotene content *B.c1.1*, suggesting that the wild allele of this gene could be relevant for the accumulation of this pigment in tomato fruits. This QTL is one of the two QTL that showed significant interactions

with the two environments analyzed here, results that suggest that the *DXS* gene could be the responsible of the variation in beta-carotene content of tomato.

Wild alleles of QTL located on chromosomes 1, 7, and 8 increased vitamin C (ascorbic acid) content in the fruits, while *S. lycopersicum* alleles of QTL on chromosome 3 seems to have also positive effect on this trait. QTL located on chromosomes 1 and 8 accounted for the largest percentage of phenotypic variance and were also involved in epistatic relationships. QTL associated with ascorbic acid content were previously reported on chromosome 2, 8, 9, 10, and 12 (Stevens et al. 2007), and most of them were unrelated to ascorbic acid metabolism genes, but associated with the expression of genes involved in pectin degradation (Di Matteo et al. 2010). However, none of these reported QTL are located in syntenic positions to those found in this study probably because different wild donor species as *S. pennelli* and *S. habrochaites* were used in those previous works. Interestingly, the *VitC8* QTL was associated to vitamin C content and co-localized with TC187, a marker for the *Tocopherol cyclase* gene. The homologous *Arabidopsis Tocopherol cyclase* gene has also been found related to ascorbic acid content (Kanwischer et al. 2005), suggesting that this gene could be an useful tool for genotyping selection of this fruit quality trait.

Conclusion

The development of a tomato genetic map based on a new RIL population allowed us to identify QTL for important fruit quality traits, which in turn largely influence the acceptance of tomato fruits in both fresh and processing markets. Our results not only confirm that the genetics of quality components in tomato is complex in nature, but also provide useful tools for genotyping and breeding purposes. This work provides evidence that QTL with main and epistatic effects as well as environment interactions are involved in the genetic variation of fruit quality traits. Some of the QTL identified here are located in similar positions to other previously reported QTL, but most of the QTL are novel and linked to codominant markers. Therefore, genetic information here reported could be useful for pyramiding several quality traits in tomato and for avoiding negative interaction with the environment, which means new chances for marker-assisted breeding of tomato crop.

Author contribution statement CC mapped most of molecular markers, performed QTL analysis, and wrote the first drafts of the manuscript under the supervision of JC and RL. AFC and AG provided and genotyped SNP markers. JMA and RF-M grew the RIL, performed the agronomic characterization of the RIL, and provided molecular markers. VL-S and MAB performed ascorbic acid determinations and provided SNP markers. FH-G and AB performed carotenoid determinations. MS and TA evaluated sugars and organic acid contents. RF-M, AG, MAB, TA, AB, and JC reviewed the manuscript. RL devised the study, wrote, and reviewed the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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