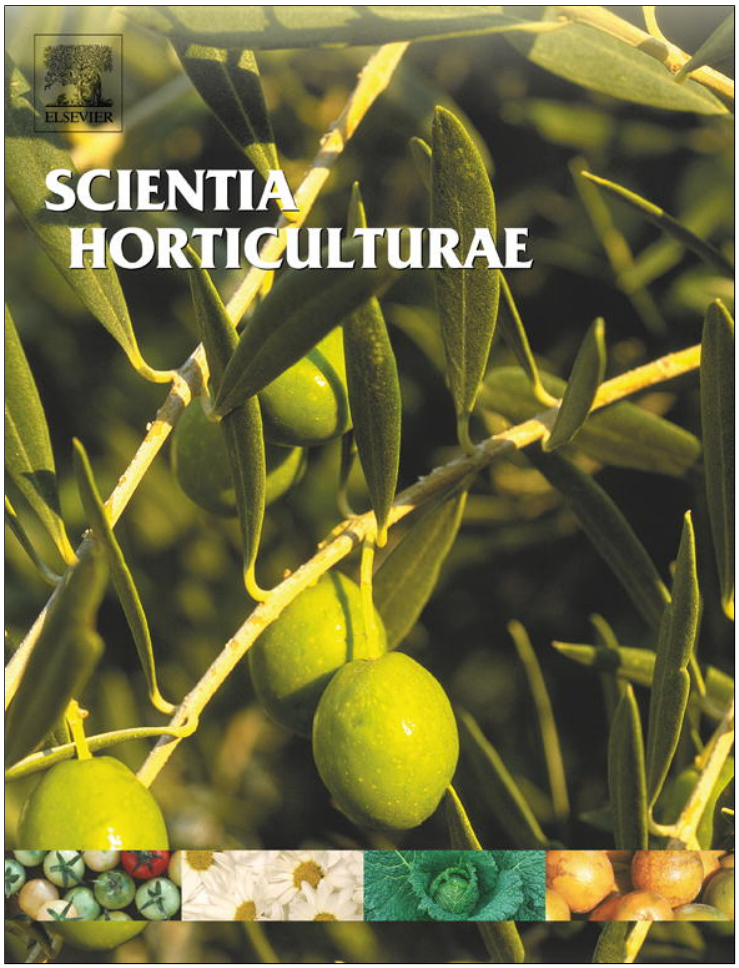

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QTL detection for fruit shelf life and quality traits across segregating populations of tomato

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ABSTRACT

The aim of this work was to detect QTLs associated with fruit shelf life and quality traits across different segregating populations derived from an interspecific cross. The first backcross generation (BC_1) of an interspecific tomato cross was analyzed to detect genomic regions from *Solanum pimpinellifolium* accession LA722 that could improve fruit quality and prolonging fruit shelf life in an Argentinean cultivar. Families derived from five BC_1 self-plant and BC_2 were used to validate the detected QTL in the BC_1 as well as to identify regions with wild type recessive alleles of QTLs controlling these traits. Thirty polymorphic markers (SSR) in parental genotypes and F_1 were used to analyze the segregating populations. The comparison among QTLs detected in the BC_1 and BC_2 generations and the families BC_1S_1 allowed assessing the consistency of six QTLs for length, shape, weight, pH, soluble solids content and fruit shelf life. QTLs with recessive effects from wild parent prolonging fruit shelf life were found and it was possible to detect QTLs for quality traits that have not been previously reported. This finding provides alternative genes for breeding programs that attempt to improve the color, soluble solids content and fruit shelf life of tomato fruits.

1. Introduction

Fruit quality in the cultivated tomato is a very important attribute to choice the appropriate cultivars by producers and also by consumers. Markets are today interested in differentiating products by attributes related to taste, aroma, acidity, sugar content and/or vitamins (Causse et al., 2002; Powell et al., 2012; Serrano-Megías and López-Nicolás, 2006). The maintenance of good organoleptic characteristics for an extended period of time after fruit harvest is defined as shelf life. A long shelf life is a desirable trait for fresh markets tomatoes. Several authors (Pereira da Costa et al., 2009; Rodríguez et al., 2006) suggested that wild tomato species are alternative genetic resources to develop long shelf life varieties without negative pleiotropic effects on other fruit quality traits. Several wild species were used in tomato quality breeding such as *Solanum pennellii* (Causse et al., 2004), *Solanum peruvianum* and *Solanum hirsutum* (Fulton et al., 2002). *Solanum pimpinellifolium* is characterized by small size and high quality fruit (Stevens and Rick, 1986) and previous studies have shown that genotypes of this wild species and its hybrids with the cultivated tomato had longer fruit shelf life with better fruit quality when compared to commercial cultivars (Rodríguez et al., 2006, 2010). Pratta et al. (2003) evaluated different tomato hybrids and concluded that the F_1 between *Solanum lycopersicum* 'Caimanta' × *S. pimpinellifolium* accession LA722 was the most promising cross for overall fruit quality improvement, because its parents had the largest differences in alleles frequencies due to the genetic divergence.

Molecular markers increase the efficiency of the introgression of genes with minor effects and furthermore allow distinguishing between genes with unfavorable effects from favorable ones (Grandillo et al., 1999). Consequently, the aim of this work was to detect QTLs associated with fruit shelf life and other quality traits in successive segregating populations obtained from an interspecific cross among an Argentinean cultivar of *S. lycopersicum* and an accession of *S. pimpinellifolium*.

2. Materials and methods

2.1. Plant material

The Argentinean cultivar Caimanta of *S. lycopersicum* (CAI) was used as recurrent parent. Caimanta has a determinate growth

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habit, compact plants, flat fruit (higher diameter than length, with an average weight of 98.5 ± 9.9 g and an average fruit shelf life of 9.7 ± 0.9 days). The donor parent was *S. pimpinellifolium* accession LA722 with indeterminate growth habit and a large number of flowers per inflorescence. This genotype has round and small fruits with an average weight of 0.9 ± 0.1 g and 18.7 ± 0.4 days of fruit shelf life (Rodríguez et al., 2006). The F_1 generation was obtained by hand crossing following the technique described by Rick (1973) and using the cultivar Caimanta as the female parent. The backcross 1 (BC_1) and backcross 2 (BC_2) generations were obtained (Fig. S1, supplementary section). In a first cycle of evaluation 80 seeds of the BC_1 generation were germinated in seedling (Table S1, supplementary section). The seedlings were transplanted to a greenhouse in a completely randomized design. The distance between plants was 35 cm and row spacing of 1 m. To assess the consistency of detected QTLs in BC_1, 50 BC_2 plants and five families BC_1S_1 (derived from BC_1 plants III-21, VII-31, VII-51, VII-91, and IX-14) were studied (Table S1, supplementary section). In both cycles of evaluation the parental genotypes and the F_1 were used as testers.

2.2. Phenotypic analysis

Ten fruits per plant at the breaker stage defined by Giovannoni (2004) were harvested and evaluated for weight (*W*, g), diameter (*D*, cm), length (*H*, cm), shape (Sh, length/diameter ratio), shelf life (SL) as days from harvest to the beginning of fruit softening, following the methodology described by Garg et al. (2008) and Rodríguez et al. (2011). The fruits were placed on shelves at 25 ± 3 °C and were discarded at 48 h interval at the first visual sign of deterioration which was a slight wrinkling of the fruit skin or excessive fruit softening. In fruits harvested at the red ripe stage defined by Giovannoni (2004), the following traits were evaluated: soluble solids content (SS, °Brix) measured with a manual refractometer, pH of the homogenized juice and titratable acidity (TA, g citric acid/100 g of homogenized juice). TA was determined by titrating 10 g of juice dissolved in 100 ml of distilled water against 0.1 N sodium hydroxide (NaOH), using a pH = 8.1 as final point. To evaluate SS, TA, and pH three independent samples composed by juice obtained from 3 to 8 fruit per plant were taken depending on fruit size.

The fruit firmness (*F*) was measured on the equatorial plane, in two opposite fruit areas with a durometer type Shore A (Durofel DFT100) with a tip of 0.10 cm^2.

The fruit color was evaluated by the darkness (*L*) and the ratio *a*/*b*. The parameters *L*, *a* (absorbance at 540 nm) and *b* (absorbance at 675 nm) were determined with a Minolta® Chromameter CR 400. The firmness and fruit color were determined in at least five fruits per plant.

The normal distribution of the traits in all generations was verified by the Shapiro–Wilk test (Shapiro and Wilk, 1965). *t*-Student test (Snedecor, 1964) was used to compare means between parents and F_1. All statistical analysis was carried out with InfoStat software Version 1.0 (Di Renzo et al., 2001).

2.3. Molecular analysis

Young leaves were removed from the parents, F_1 and every plant of BC_1, BC_2 and BC_1S_1 generations, which were kept in −80 °C freezer until DNA extraction. A commercial kit (Wizard® Genomic DNA Purification Kit from Promega®) was used. Amplifications were done by duplicate for each parent and the F_1 genotype.

Fifty-five DNA molecular markers type SSR (single sequence repeats) covering the genome were tested in parental genotypes and the F_1. These SSRs were chosen due to their codominant nature and their chromosome location. The sequence of primers, size of the cultivated and wild alleles and chromosomal location was obtained from http://www.solgenomics.net (Table S2, supplementary section). Polymorphic markers distributed in the tomato genome and located preferably on different arms were chosen. SSRs that showed polymorphism among the parental genotypes and F_1 were subsequently used for genotyping the BC_1, BC_2, and families BC_1S_1. For PCR reaction, the standard protocol was followed (Powell et al., 1996). Separation of amplification fragments was carried out in polyacrylamide gels (6%, w/v) at room temperature. The visualization was performed by commercial silver staining kit (Silver Sequence™ Staining Regeants, Promega®).

Given the codominant nature of SSR markers, we tested the expected Mendelian segregation in the BC_1 and BC_2 generations (1:1, homozygous:heterozygous) and in the BC_1S_1 (1:2:1), through a χ^2 test (Snedecor, 1964).

Association between molecular markers and traits was determined by single point analysis (Tanksley, 1993) because a complete linkage map is not required (Collard et al., 2005). Consequently one-way ANOVA was performed, in which genotype at marker loci was used as the classifying variable. When more than two genotypes at marker loci were analyzed, comparisons among their mean phenotypic values for the corresponding trait were done by Fisher's least significant differences (LSD). A p-value < 0.001 was chosen as threshold to define the association between a QTL and a marker locus (p-value < 0.001 corresponds to a LOD-score of 2.4, Grandillo and Tanksley, 1996). However, QTLs detected with p-value < 0.05 were also scored, since their consistency could be evaluated in the second cycle when BC_1S_1 and BC_2 generations were analyzed (Li et al., 2011). The R^2 values were used to estimate the percentage of total phenotypic variation explained by each QTL (Liu, 1998). The degrees of dominance (d/a) were calculated for each QTL detected from three possible genotypes and phenotypic data for this QTL in the BC_1S_1 families.

3. Results

3.1. Phenotypic analysis in the parents and the segregating generations

The parental genotypes were significantly different for all traits analyzed, except for soluble solids content and pH (Table 1).

Broad variation ranges were observed for most of traits in each family (Table 2). Plants with yellow fruit appeared in the segregation of families III-21, VII-31, VII-51 and VII-91. All families had high values for soluble solids content, similar to LA722 parent.

3.2. Molecular analysis in BC_1 generation

Thirty of the 50 SSR markers were polymorphic among parents and F_1 and then tested in the BC_1 generation. Twelve SSR (40%) showed skewed segregation from the expected Mendelian ratio. From the association analysis, 42 QTLs were significant at 5%. Some of these associations, 17 (40%) were significant at 1% and six (14%) at 0.1%. The associations at 0.1% are highlighted in bold in Table 3. In average 3.5 QTLs per chromosome and 3.8 QTLs per trait were detected. QTLs for fruit shelf life were not found in the analysis of this segregating generation. Seven QTLs were found for fruit weight, which was the trait with the highest number of phenotypic/molecular associations. On chromosomes 9 and 10 the highest number of QTLs (seven in each one) was found, whereas on chromosome 5 only one QTL associated to pH was found. The percentage of phenotypic variation explained by each marker varied among 6% and 18% and the highest R^2 value was for a QTL that controls fruit diameter located on chromosome 8. Fifty percent of detected QTLs (21) accounted for less than 10% of phenotypic variation.

Table 1
Mean values and standard error (SE) for each evaluated trait of cv Caimanta of *Solanum lycopersicum* (CAI), *S. pimpinellifolium* accession LA722 (LA722) and the F_1. Mean values and standard deviation (SD) for each trait in the BC_1 generation.

Traits	Genotypes				
	CAI Mean ± SE	LA722 Mean ± SE	F_1 Mean ± SE	BC_1	
				Mean	SD
Diameter (cm)	6.46 ± 0.28c	1.22 ± 0.03a	2.17 ± 0.03b	3.50	0.61
Length (cm)	3.99 ± 0.14c	1.13 ± 0.02a	2.02 ± 0.03b	2.97	0.46
Shape	0.65 ± 0.03a	0.95 ± 0.03b	0.93 ± 0.01b	0.86	0.08
Weight (g)	97.84 ± 11.1c	1.17 ± 0.08a	6.21 ± 0.20b	23.63	9.81
Shelf life (days)	7.96 ± 0.63a	15.26 ± 0.60b	16.39 ± 0.37b	13.53	4.18
Firmness	35.88 ± 3.52a	65.61 ± 1.40c	49.30 ± 1.97b	49.71	8.22
Darkness (L)	41.10 ± 1.92c	38.52 ± 0.21b	36.84 ± 0.32a	41.04	1.92
Ratio *a*/*b*	0.94 ± 0.04a	1.42 ± 0.02b	1.34 ± 0.03b	1.02	0.12
Soluble solids content (°Brix)	7.52 ± 0.03a	7.25 ± 0.35a	8.73 ± 0.08b	6.27	0.88
pH	4.79 ± 0.03a	4.83 ± 0.06a	4.76 ± 0.04a	4.68	0.22
Titratable acidity (g)	0.26 ± 0.01a	0.91 ± 0.03c	0.43 ± 0.01b	0.31	0.09

Different letters indicate significant differences ($p < 0.05$) according to a *t*-test.

3.3. Molecular analysis in BC_1S_1 and BC_2 generations

In order to detect recessive alleles of QTL from LA722, BC_1S_1 families were evaluated. These BC_1S_1 were obtained from a set of five BC_1 plants (III-21, IX-14, VII-31, VII-51, and VII-91). These plants were chosen because they had long shelf life and they were also heterozygous for all the 30 SSR markers considered. The criteria of selecting these plants assured the segregation of the phenotypic trait as well as the 30 SSR markers. Moreover, these BC_1S_1 families together with 50 BC_2 plants were used to assess the consistency of those putative QTLs detected in the BC_1 generation.

Only three markers had distorted segregation when the five families BC_1S_1 were evaluated.

In the BC_1S_1 families a mean value of 12 QTLs ($p < 0.05$) per family were detected. However, 10% (six QTLs) had highly significant effects on fruit quality traits ($p < 0.001$) (Table 4A–E, highlighted in bold). The most significant QTL was associated with pH and other QTL for length, diameter, shape, and fruit firmness, respectively. The SSR327 marker, located on chromosome 8, was associated with fruit shelf life trait in the BC_1S_1 (VII-31) family (Table 4C). The presence of this marker explained 18% of the phenotypic variation of the trait with the LA722 alleles increasing the mean value of the trait meanwhile the cultivated allele decreased the mean value of it. In the BC_1S_1 (VII-51) family, two QTLs that explained 18% and 22% of the phenotypic variation for fruit shelf life were detected on the chromosomes 10 and 11, respectively (Table 4D). The LA722 allele of the QTL on Chr 10 increased the mean value of the trait, while for the QTL on Chr 11 the heterozygous individuals had higher mean values of fruit shelf life, showing some heterotic effect at this locus. In addition, in the BC_1S_1 (VII-91) family the fruit shelf life was explained by two QTLs located on chromosomes 1 and 10 respectively (Table 4E). Also, it was found a heterotic effect for the QTL on Chr10 while for the QTL on Chr 1 the cultivated allele increased the mean value of this trait.

Two markers located on chromosome 10 (SSR596 and SSR318) were associated with fruit shelf life in the BC_2 generation ($p < 0.05$) (data not shown). In both markers the wild type allele increased the mean of the trait.

3.4. Consistency of the QTLs across backcrosses and selfed generations

The associations found in the BC_1 and BC_2 generations and in the five selfed families were compared for their consistency. This comparison allowed validating 11 QTLs for length, shape, weight, pH, soluble solids content and fruit shelf life, which are shown in Table 5.

The comparison between families from self-pollination of BC_1 plants allowed the detection of two markers (SSR034 and SSR038) associated with the same attributes in at least two families (Table 5). These markers showed association with color index (*a*/*b*), titratable acidity (TA) and soluble solids content (SS). SSR288 associated with pH, SSR038 associated with *a*/*b*, and SSR034 associated with both TA and fruit shape but they did not have the same effect on those traits in each generation in which they were detected.

Table 2
Mean values and standard deviations (SD) for each fruit quality trait in BC_1S_1 families.

Traits	BC_1S_1 families									
	III-21		IX-14		VII-31		VII-51		VII-91	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Diameter (cm)	3.41	0.63	3.72	0.44	2.95	0.45	3.51	0.48	2.76	0.48
Length (cm)	2.93	0.46	3.02	0.33	2.77	0.38	2.88	0.36	2.48	0.43
Shape	0.87	0.05	0.82	0.04	0.95	0.05	0.82	0.05	0.91	0.05
Weight (g)	23.42	10.64	26.76	7.76	15.56	5.96	22.73	8.34	13.38	6.55
Shelf life (days)	11.91	4.11	11.50	2.22	13.19	3.67	10.95	3.38	13.38	4.77
Firmness	57.53	9.11	55.52	6.04	58.40	9.74	57.24	7.99	54.41	8.44
Darkness (L)	40.32	5.67	37.29	1.30	43.50	8.72	43.93	8.05	43.49	9.25
Ratio a/b	1.09	0.35	1.26	0.09	0.80	0.61	0.89	0.47	0.95	0.60
Soluble solids content (°Brix)	7.01	1.35	6.19	0.99	6.74	1.13	6.92	1.06	7.23	1.25
pH	4.44	0.18	4.42	0.18	4.53	0.31	4.33	0.15	4.33	0.26
Titratable acidity (g)	0.45	0.15	0.45	0.11	0.42	0.13	0.58	0.16	0.58	0.17

Table 3
Fruit quality traits associated with SSR (simple sequence repeats) markers in the BC_1 generation between cv. Caimanta of *S. lycopersicum* (recurrent parent) and *S. pimpinellifolium* accession LA722.

Marker	Chr	Traits	Mean C[a]P[b]	*n*[c]CP	Mean CC	*n*CC	R^2	*p*-Value
*SSR*095	1	pH	4.73	26	4.62	23	0.06	0.0226
*SSR*288	1	pH	4.62	22	4.73	27	0.07	0.0240
*SSR*032	2	Ratio *a*/*b*	0.98	18	1.04	33	0.06	0.0436
		Titratable acidity (g)	0.27	18	0.33	33	0.12	0.0025
*SSR*598	2	Ratio *a*/*b*	0.98	18	1.04	33	0.06	0.0436
		Titratable acidity (g)	0.27	18	0.33	33	0.12	0.0024
*SSR*601	3	Firmness	53.72	19	47.33	32	0.14	**0.0009**
*SSR*014	3	Firmness	51.14	16	46.36	33	0.07	0.0212
*SSR*320	3	Firmness	53.72	19	47.33	32	0.14	**0.0009**
*SSR*162	5	pH	4.75	21	4.61	26	0.10	0.0067
*SSR*211044	6	Firmness	52.06	26	47.47	24	0.08	0.0170
		Darkness (L)	41.54	26	40.60	24	0.06	0.0344
*SSR*276	7	Darkness (L)	40.34	24	41.67	27	0.12	0.0024
*SSR*286	7	Ratio *a*/*b*	0.99	28	1.06	22	0.07	0.0208
*SSR*038	8	Diameter (cm)	3.21	33	3.73	40	0.18	**0.0002**
		Length (cm)	2.83	33	3.09	40	0.08	0.0154
		Shape	0.89	33	0.84	40	0.11	0.0039
		Weight (g)	19.27	33	27.29	40	0.17	**0.0004**
*SSR*327	8	pH	4.73	27	4.61	22	0.08	0.0145
*SSR*070	9	Diameter (cm)	3.72	30	3.35	44	0.09	0.0099
		Shape	0.84	30	0.88	44	0.07	0.0228
		Weight (g)	27.03	30	21.31	44	0.08	0.0128
		Ratio *a*/*b*	1.06	21	0.99	30	0.10	0.0058
		Titratable acidity (g)	0.34	22	0.28	29	0.10	0.0062
*SSR*069	9	pH	4.61	18	4.73	27	0.06	0.0375
		Titratable acidity (g)	0.34	18	0.29	29	0.10	0.0065
*SSR*318	10	Diameter (cm)	3.25	33	3.68	38	0.12	0.0027
		Length (cm)	2.81	33	3.10	38	0.11	0.0056
		Weight (g)	19.39	33	29.89	38	0.15	**0.0009**
*SSR*596	10	Diameter (cm)	3.27	31	3.63	41	0.09	0.0107
		Length (cm)	2.76	31	3.11	41	0.14	0.0010
		Weight (g)	19.26	31	26.19	41	0.12	0.0019
*SSR*034	10	Shape	0.83	34	0.89	36	0.16	**0.0007**
*SSR*G036	11	Diameter (cm)	3.65	37	3.34	37	0.07	0.0274
		Length (cm)	3.09	37	2.85	37	0.07	0.0247
		Weight (g)	26.14	37	21.11	37	0.07	0.0263
*SSR*080	11	Soluble solids content (°Brix)	6.59	19	6.09	34	0.08	0.0454
*SSR*220060	11	Weight (g)	21.03	39	25.95	33	0.07	0.0303
*SSR*H301	12	Diameter (cm)	3.10	13	3.59	58	0.10	0.0089
		Weight (g)	16.3	13	25.30	58	0.12	0.0025
		pH	4.79	9	4.65	37	0.07	0.0251
		Titratable acidity (g)	0.25	10	0.32	38	0.13	0.0023

Associations with $p < 0.001$ are highlighted in bold.
[a] Caimanta allele.
[b] LA722 allele.
[c] Number of plants.

4. Discussion

Fruit weight QTLs have been well studied in tomato. Lippman and Tanksley (2001) found major QTLs located on chromosomes 1, 3 and 11 which explained 67% of the fruit weight phenotypic variation and the wild type alleles always decreased the mean value for the trait. In this experiment, a wild type allele for a QTL on chromosome 9 detected in the BC_1 generation (Table 3, $p < 0.001$) increased the fruit diameter. Molecular markers allowed us to identify some wild type alleles that increase fruit size in individuals with unfavorable phenotypes.

Based on the color ideotype proposed by Sacks and Francis (2001), *S. pimpinellifolium* LA722 accession having high a/b and low *L* values would be a good novel material for a breeding program which aims to improve the fruit color. Only one QTL for *a*/*b* (SSR038, Chr 8) with negative effect was validated among BC_1S_1 families (Table 5).

In this experiment two markers were associated with firmness, both being detected on chromosome 3 (Table 3). In agreement with these findings, Tanksley et al. (1996) found four QTLs for fruit firmness located on chromosome 3 verifying that the wild type alleles had significant and positive effect on this trait.

Tanksley and Nelson (1996) proposed the advanced backcross method using families BC_2S_2 or BC_2S_1 as a strategy to detect recessive alleles of QTLs donors in addition to the alleles of QTLs with additive and dominant effects. In the association analysis of SSR markers with phenotypic traits in the five BC_1S_1 progenies, new QTLs were found for pH, length, diameter, shape and fruit firmness ($p < 0.001$) (Table 4A–E). Recombination during self-pollination could produce different genetic backgrounds for the QTLs that modify the expression of them or perhaps this differential expression also could be due to environmental effects.

We validated the association between SSR034 (Chr10) and soluble solids content in two BC_1S_1 families and its effect on this trait

Table 4
Fruit quality traits associated with SSR (simple sequence repeats) markers in family BC_1S_1 (III-21) (A), family BC_1S_1 (IX-14) (B), family BC_1S_1 (VII-31) (C), family BC_1S_1 (VII-51) (D) and family BC_1S_1 (VII-91) (E).

Trait	Marker	Chr	Mean CC	Mean CP	Mean PP	LSD[c]	R^2	*p*-Value	d/a[a]
(A) BC_1S_1 (III-21)									
Soluble solids content (°Brix)	*SSR*598	2	5.84a	7.29b	7.50b	0.92	0.26	0.0032	0.75
pH	*SSR*598	2	4.25a	4.52b	4.50b	0.12	0.38	**0.0001**	1.16
Soluble solids content (°Brix)	*SSR*032	2	6.11a	7.29b	7.50b	0.97	0.19	0.0180	0.70
pH	*SSR*032	2	4.27a	4.52b	4.50b	0.12	0.35	**0.0002**	1.17
Darkness (L)	*SSR*115	5	43.64b	39.19a	37.88a	4.02	0.19	0.0183	−0.55
Ratio *a*/*b*	*SSR*115	5	0.87a	1.15b	1.27b	0.25	0.21	0.0093	0.40
pH	*SSR*128	6	4.52b	4.49b	4.32a	0.14	0.21	0.0090	0.70
Titratable acidity (g)	*SSR*034	10	0.36a	0.45a	0.55b	0.11	0.23	0.0064	−0.05
Darkness (L)	*SSR*080	11	44.47b	38.65a	40.43ab	4.31	0.15	0.0423	nc[b]
(B) BC_1S_1 (IX-14)									
Weight (g)	*SSR*295	2	29.89b	28.00b	22.35a	5.37	0.14	0.0391	0.50
Soluble solids content (°Brix)	*SSR*295	2	5.81a	6.14ab	6.85b	0.71	0.14	0.0429	−0.37
Soluble solids content (°Brix)	*SSR*327	8	6.93b	5.98a	5.56a	0.67	0.27	0.0013	−0.39
Titratable acidity (g)	*SSR*327	8	0.52b	0.44a	0.37a	0.08	0.23	0.0044	−0.07
Ratio *a*/*b*	*SSR*038	8	1.29b	1.20a	1.31b	0.06	0.24	0.0028	nc[b]
Titratable acidity (g)	*SSR*038	8	0.52b	0.40a	0.43a	0.07	0.26	0.0020	−1.67
Soluble solids content (°Brix)	*SSR*344	8	5.37a	6.47b	6.26ab	0.77	0.16	0.0276	nc
Titratable acidity (g)	*SSR*344	8	0.38a	0.46ab	0.51b	0.08	0.15	0.0389	0.23
Titratable acidity (g)	*SSR*034	10	0.36a	0.44a	0.55b	0.08	0.24	0.0021	−0.16
Weight (g)	*SSR*220060	11	29.34b	28.25b	21.21a	5.83	0.16	0.0280	0.73
Soluble solids content (°Brix)	*SSR*220060	11	6.44ab	5.80a	6.46b	0.58	0.18	0.0168	nc
(C) BC_1S_1 (VII-31)									
Shape	*SSR*128	6	0.98b	0.94a	0.93a	0.04	0.17	0.0424	−0.60
Shape	*SSR*286	7	1.00b	0.96b	0.91a	0.04	0.31	0.0033	0.11
Soluble solids content (°Brix)	*SSR*286	7	7.92b	6.62a	6.47a	1.00	0.18	0.0449	−0.79
Ratio *a*/*b*	*SSR*038	8	1.14b	0.74ab	0.21a	0.52	0.20	0.0229	0.14
Shelf life (days)	*SSR*327	8	12.00a	12.47a	15.70b	2.81	0.18	0.0331	−0.75
Diameter (cm)	*SSR*063	8	3.18a	2.98a	2.68a	0.36	0.17	0.0451	0.20
Ratio *a*/*b*	*SSR*063	8	1.13b	0.78ab	0.42a	0.47	0.19	0.0303	0.01
Length (cm)	*SSR*220060	11	3.02b	2.72a	2.55a	0.32	0.18	0.0409	−0.28
Soluble solids content (°Brix)	*SSR*080	11	6.38a	6.43a	7.54b	0.91	0.22	0.0182	−0.91
Length (cm)	*SSR*H301	12	2.78ab	2.91b	2.48a	0.30	0.20	0.0227	nc[b]
(D) BC_1S_1 (VII-51)									
Soluble solids content (°Brix)	*SSR*288	1	6.47a	6.65a	7.78b	0.84	0.26	0.0101	−0.73
pH	*SSR*288	1	4.23a	4.34ab	4.41b	0.12	0.19	0.0391	0.22
Diameter (cm)	*SSR*045	7	3.06a	3.80b	3.27a	0.34	0.47	**0.0003**	nc[b]
Length (cm)	*SSR*045	7	2.69a	3.06b	2.66a	0.30	0.30	0.0097	nc
Weight (g)	*SSR*045	7	16.74a	27.54b	17.56a	6.25	0.41	0.0011	nc
Shape	*SSR*045	7	0.89b	0.80a	0.82a	0.05	0.38	0.0019	−1.57
Diameter (cm)	*SSR*034	10	4.03b	3.38a	3.34a	0.41	0.36	0.0044	−0.88
Length (cm)	*SSR*034	10	3.28c	2.86b	2.59a	0.28	0.48	**0.0004**	−0.22
Weight (g)	*SSR*034	10	31.91b	21.41a	18.02a	7.13	0.38	0.0029	−0.51
Shape	*SSR*034	10	0.82ab	0.84b	0.78a	0.05	0.22	0.0495	nc
Soluble solids content (°Brix)	*SSR*034	10	6.01a	7.57b	7.00ab	0.99	0.31	0.0125	nc
Length (cm)	*SSR*596	10	3.12b	2.81a	2.75a	0.30	0.18	0.0476	−0.68
Shelf life (days)	*SSR*596	10	10.19a	10.01a	13.27b	2.79	0.18	0.0480	−1.12
Shelf life (days)	*SSR*080	11	10.52ab	12.53b	8.72a	3.10	0.22	0.0483	nc
(E) BC_1S_1 (VII-91)									
Diameter (cm)	*SSR*095	1	3.24b	2.69a	2.56a	0.34	0.29	0.0017	−0.62
Length (cm)	*SSR*095	1	2.83b	2.48a	2.27a	0.32	0.21	0.0110	−0.25
Weight (g)	*SSR*095	1	19.4b	12.68a	10.85a	4.73	0.23	0.0062	−0.57
Soluble solids content (°Brix)	*SSR*095	1	8.01b	6.80a	7.19ab	0.93	0.15	0.0478	nc[b]
Shelf life (days)	*SSR*288	1	16.27b	12.82a	10.42a	3.42	0.21	0.0115	−0.18
Ratio *a*/*b*	*SSR*288	1	0.51a	1.09b	1.24b	0.43	0.22	0.0085	0.59
Firmness	*SSR*288	1	60.43c	53.58b	46.18a	5.75	0.32	**0.0007**	0.04
Titratable acidity (g)	*SSR*162	5	0.60a	0.55a	0.80b	0.15	0.24	0.0113	nc
Firmness	*SSR*128	6	48.87a	55.38b	51.94ab	5.48	0.25	0.0163	nc
Shelf life (days)	*SSR*034	10	10.47a	14.79b	11.23a	3.53	0.18	0.0228	nc
Firmness	*SSR*034	10	53.4ab	56.75b	47.55a	6.25	0.18	0.0096	nc
Soluble solids content (°Brix)	*SSR*034	10	7.02a	7.02a	8.13b	0.96	0.15	0.0456	−1.00
Length (cm)	*SSR*1061	11	2.62b	2.59b	2.11a	0.37	0.21	0.0340	0.88
Shape	*SSR*1061	11	0.89a	0.94b	0.86a	0.04	0.40	**0.0008**	nc
Firmness	*SSR*1061	11	60.89b	52.90a	50.05a	7.77	0.21	0.0363	−0.47

Different letters indicate significant differences ($p < 0.05$) between homozygous individuals for Caimanta alleles (CC), homozygous individuals for LA722 alleles (PP) and heterozygous individuals (CP). Associations with $p < 0.001$ are highlighted in bold.

[a] Degree of dominance.
[b] Not calculated.
[c] LSD Fisher value at $\alpha = 0.05$.

Table 5
Consistency of found associations among BC_1, BC_2, and BC_1S_1 (A) and among families (B) and effect of the wild type alleles on the phenotypic value.

	Trait	Marker	Chr	Generation	*p*-Value	Effect of LA722 allele
A	Length (cm)	*SSR*596	10	BC_1	0.0010	Decrease
				BC_1S_1 (VII-51)	0.0476	Decrease
				BC_2	0.0483	Decrease
		*SSR*318	10	BC_1	0.0056	Increase
				BC_2	0.0483	Decrease
		*SSR*220060	11	BC_1S_1 (VII-31)	0.0409	Decrease
				BC_2	0.0023	Decrease
	Shape	*SSR*034	10	BC_1	0.0007	Decrease
				BC_1S_1 (VII-51)	0.0495	[a]
	Weight (g)	*SSR*220060	11	BC_1	0.0303	Decrease
				BC_1S_1 (IX-14)	0.0280	Decrease
				BC_2	0.0067	Decrease
	pH	*SSR*288	1	BC_1	0.0240	Decrease
				BC_1S_1 (VII-51)	0.0391	Increase
	Soluble solids content (°Brix)	*SSR*080	11	BC_1	0.0454	Increase
				BC_1S_1 (VII-31)	0.0182	Increase
	Shelf life (days)	*SSR*596	10	BC_2	0.0470	Increase
				BC_1S_1 (VII-51)	0.0480	Increase
B	Ratio *a*/*b*	*SSR*038	8	BC_1S_1 (IX-14)	0.0028	[a]
				BC_1S_1 (VII-31)	0.0229	Decrease
	Titratable acidity (g)	*SSR*034	10	BC_1S_1 (III-21)	0.0064	Increase
				BC_1S_1 (IX-14)	0.0021	Increase
	Soluble solids content (°Brix)	*SSR*034	10	BC_1S_1 (VII-51)	0.0125	[a]
				BC_1S_1 (VII-91)	0.0456	[a]

[a] Heterozygous genotypes showed significant differences with both homozygous genotypes.

was consistent in both families (Table 5). The results indicate the genuine nature of this QTL and their potential value for SS breeding in tomato via marker assisted selection.

In two SSRs associated with fruit shelf life (SSR327 on Chr8 and SSR596 on Chr10, Table 4C and D), the LA722 allele increased the mean value, but its effect was recessive with respect to CAI allele, which would explain why it was not detected in BC_1. Association of SSR596 with fruit shelf life was consistent in BC_1S_1 and BC_2 generations being this QTL a new genetic source for breeding programs that attempt to extend this trait.

In the BC_2 generation the presence of a QTL for fruit shelf life is remarkable since it had not been detected in the BC_1 generation likely due to different genetic backgrounds in both generations (BC_1 and BC_2).

Other two QTLs for fruit shelf life were found (Table 4D and E), but in this case heterozygous individuals had longer shelf life than homozygous individuals for CAI and LA722 alleles. This observation would indicate some intralocus interaction (heterotic effect). Finally, the cultivated alleles in a QTL on chromosome 1, prolonged the fruit shelf life (Table 5E). These results demonstrate the presence of favorable alleles for this trait in the cultivated genotype as it has been previously reported by Pratta et al. (2011).

Evaluating BC_1S_1 generations was useful to detect QTLs whose wild type alleles have recessive effect. Some QTLs for fruit shelf life are like this kind. This QTL analysis provides strong evidence that there are several loci involved in the control of fruit shelf life. Additional experiments should be conducted to better understand the individual effect and the interaction of these fruit shelf life QTLs. Furthermore, the use of a higher number of markers and extra segregating populations could allow us to have a best coverage of the genome and probably detect extra minor QTLs underlying this trait. Nevertheless, the fruit shelf life loci reported in this study offer a new background of genes that prolong the shelf life without provoking adverse effects on quality traits.

5. Conclusion

The presence of markers linked to fruit quality traits hereby detected show that there are undiscovered genetic variability that has not been sufficiently explored. Broad interspecific crosses would be most appropriate for traits that have been neglected during domestication process and it can be recovered by introgression in breeding programs. QTLs with recessive effects from wild parent that prolong fruit shelf life were found. In this work we have detected QTLs for quality traits that have not been previously reported, providing alternative genes for breeding programs that attempt to improve the color, soluble solids content and shelf life of tomato fruits.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.scienta.2013.03.015.

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