Identification and characterization of quantitative trait loci related to chemical traits in tomato (*Lycopersicon esculentum* Mill.)

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ABSTRACT

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Traits such as soluble solids content, fruit acidity, pH, fruit lycopene concentration, fruit beta-carotene concentration, and fruit xanthophyll concentration are very important to the tomato industry. In this study, quantitative trait loci (QTL) analysis of these traits was performed using a recombinant inbred line (RIL) population derived from a cross between a tomato (*Lycopersiconesculentum Mill*)line, NC84173, and an inbred accession, LA722 (*Lycopersicon pimpinellifolium*).The two parental lines and the 116 RILs were planted in an alpha-lattice design with two replications. At the end of the growing season, 30-50 representative ripened fruits were harvested randomly from each RIL and the parents to record the traits. A linkage map was constructed using 121RFLP and 67RGA markers arranged in 12 groups. Transgressive segregation was observed for all the traits. A total of 10 quantitative trait loci (QTLs) were detected for the six traits using composite interval mapping. Co-localization of QTLs for lycopene, beta-carotene, and xanthophyll may reflect the pleiotropy effect or genetic linkage between genes via physiological relationships among these traits. This analysis shows that the introgression of wild germplasm may improve the nutritional quality of tomatoes.

Keywords: chemical traits, QTL, composite interval mapping, tomato

INTRODUCTION

Tomatoes (*Lycopersicon esculentum* Mill.) are among the most popular vegetable crops in the world and play a key role in the humandiet. The nutritional importance of tomatoes is related to their chemical composition (Brandt *et al*., 2006). Tomatoes are a rich source of carotenoids. Lycopene is the major carotenoid present in tomatoes, accounting for >80% of all carotenoids in fully red-ripe fruit, where it is responsible for their characteristic color (Nguyen and Schwartz, 1999).

Tomatoes also contain moderate amounts of beta-carotene, which is known for its provitaminA activity (DiMascio *et al.,* 1991).The antioxidant capacity of lycopene is at least twice that of beta-carotene (Di Mascio *et al.,* 1991). Other carotenoids present in ripe tomato fruits include small amounts of phytoene, phytofluene, neorosporene, and lutein (Khachik *et al.,* 2002). Moreover, several important changes occur in the ultra-structure of tomatoes during ripening. For example, Giovanelli *et al.* (1999) reported an increase in the ascorbic acid content of tomatoes during ripening.

Development of tomato cultivars with improved fruit using traditional breeding approaches requires an efficient screening method. During the past several years, DNA-based markers have been used successfully to map loci controlling quantitative trait variation. Quantitative trait loci (QTL) analysis can be used to dissect genetic variance in populations derived from crosses both within species, as well as between elite and exotic or wild germplasm (Bernacchi *et al.,* 1998). Tomato is one of the first plant species inwhich researchers began to map QTLs of agronomic traits using molecular markers (Atherton and Harris, 1986).

In previous studies, a variety of beneficial QTLs for soluble solids content (Bernacchi *et al.,* 1998; Fulton *et al.*, 2000, Monforte *et al.,* 2001; Paterson *et al*., 1991), lycopene content (Brandt *et al*., 2006), fruit shape (Fulton *et al*., 2000; Monforte *et al.,* 2001), acid content and fruit pH (Fulton *et al.,* 2000;

Paterson *et al.,* 1991), fruit size and shape (Grandillo *et al.,* 1999), and lycopene and beta-carotene content (Rousseaux *et al.,* 2005) have been mapped in tomato. However, additional experiments to verify the presence and effects of these QTLs are needed before attempts are made forgene cloning and applicationin marker-assisted selection breeding program.

The wild relatives of tomato are a rich source of genes for many of tomato's chemical traits (Chen *et al.,* 1999). *Lycopersicon pimpinellifolium*is arguably the most closely related wild species of tomato (Rick, 1983) and therefore is suitable germplasm for tomato improvement. In this study, we used an accession (LA722) of *L. pimpinellifolium* that exhibited a number of desirable horticultural characteristics including high levels of lycopene and high soluble solids content (Chen *et al.,* 1999), and cold and salt tolerance during seed germination (Foolad *et al.,* 1998a,b).

The aim of the present study was to identify genomic regions controlling fruit acidity, pH, soluble solids content, fruit lycopene concentration, fruit beta-carotene concentration, and fruit xanthophyll concentration in a RIL population derived from a cross between a breeding line, NC84173, and an inbred accession, LA722.

MATERIALS AND METHODS

Plant materials

One hundred and sixteen recombinant inbred lines (F10) derived from a cross between a horticulturally superior, multiple disease resistant, fresh-market tomato breeding line (R.Gardner, North Carolina State University, Fletcher,NC), NC84173 (*Lycopersicon esculentum* Mill.), and a self-compatible, inbred accession, LA722 (*L. pimpinellifolium*) were used in this study (Foolad *et al.,* 1998a).

Trait measurement

The two parental lines, NC84173 and LA722, and the 116 RILs were planted in 2005 at the experiment station of the College of Agriculture of the University of Tehran, Iran. The field experiment was conducted in analpha-lattice design with two replications, using row × plant spacing of 150 cm × 100 cm. At the end of the growing season, 30-50 representative ripened fruits were randomly harvested from each plot and evaluated for the following traits in two replications.

Total soluble solids content (SSC): The mixed fresh juice of 20-30 fruits per plot (homogenized in ablender) was used to measure SSC in °Brix, using ATAGO refractometers (Spectronic Instruments, Inc., Rochester, NY).

Fruit pH: This was measured with a pH meter on the same fruit juice sample used for solids measurements.

Fruit acidity: Acidity was determined by titration of the homogenized juice sample with 25 gL^{-1} NaOH.

Fruit carotenoidcontent: Carotenoid content was determined using aspectrophotometer, as described by Bicanic *et al.* (2004), with minor modifications. Before actual analysis, the samples were equilibrated overnight at room temperature. Around 150-200 g of each sample washomogenized in a shaker. Then 50 mL of a solvent mixture (hexan/acetone/abs EtOH, 2:1:1) was added after adding 4 mL water and the samples were shaken for 10 min. After adding 7.5 mL of water, the shaking was continued for another 5 minutes. The layers separated, and the deeply colored hexane layer was diluted (10 to 100 times) with hexane. The spectrum of the fresh hexane solution containing lycopene, beta-carotene, and xanthophyllwas recorded, and absorbance at 480, 460, and 450 nm was measured for lycopene, beta-carotene, and xanthophylls, respectively, using a Minolta Spectrophotometer. Each sample product was analyzed in two replications and the concentration of each carotenoid determined in ppm.

Map construction and QTL mapping:

The map was constructed by Dr. Foolad, Department of Horticulture, The Pennsylvania State University using 121 RFLP and 67 RGA markers (Foolad *et al.,* 1998a, b). QTL analysis was performed using composite interval mapping while threshold LOD was 2.5 with 2 cM in each step through QTL-Cartographer V. 1.15 (Christopher *et al*., 2001).

RESULTS

Phenotypic variation

The maximum, minimum, mean, and standard deviation of the six traits of the RIL population and their parents are shown in Table 1. For all traits except pH, the differences between the two parents were significant. Moreover, the ranges in the values for all traits exceeded those of the parents, and transgressive phenomena were prominent for all traits. Compared with NC84173, LA722 showed higher values for all traits except pH.

Map construction and QTL analysis

A genetic linkage map was constructed based on 90 RFLP and 28 RGA markers and 116 RI lines (Fig. 1). This map spanned 1806 cM with an average interval length of 9.6 cM between markers. The number of markers per chromosome ranged from 7 (chromosome 11) to 25 (chromosome 1). Chromosome 1 had the largest linkage group (224 cM), followed by chromosomes 3 and 9 (220 and

Table 1. Variation of studied traits in the recombinant inbred line (RIL) population derived from *Lycopersicon esculentum* and *L. pimpinellifolium*.

Trait	Parents		RIL population			
	NC84173	LA722	Mean	Max.	Min.	SD
pH	4.17	3.85	4.12	4.50	3.80	0.12
Acidity	3.57	11.76	6.65	9.97	2.63	1.45
Soluble solids	3.85	7.80	6.32	9.20	3.60	1.04
Lycopene	30.36	69.12	69.69	148	17.36	20.20
Beta-carotene	0.22	0.45	0.45	0.97	0.16	0.12
Xanthophyll	1.75	3.58	3.69	7.80	0.65	1.02

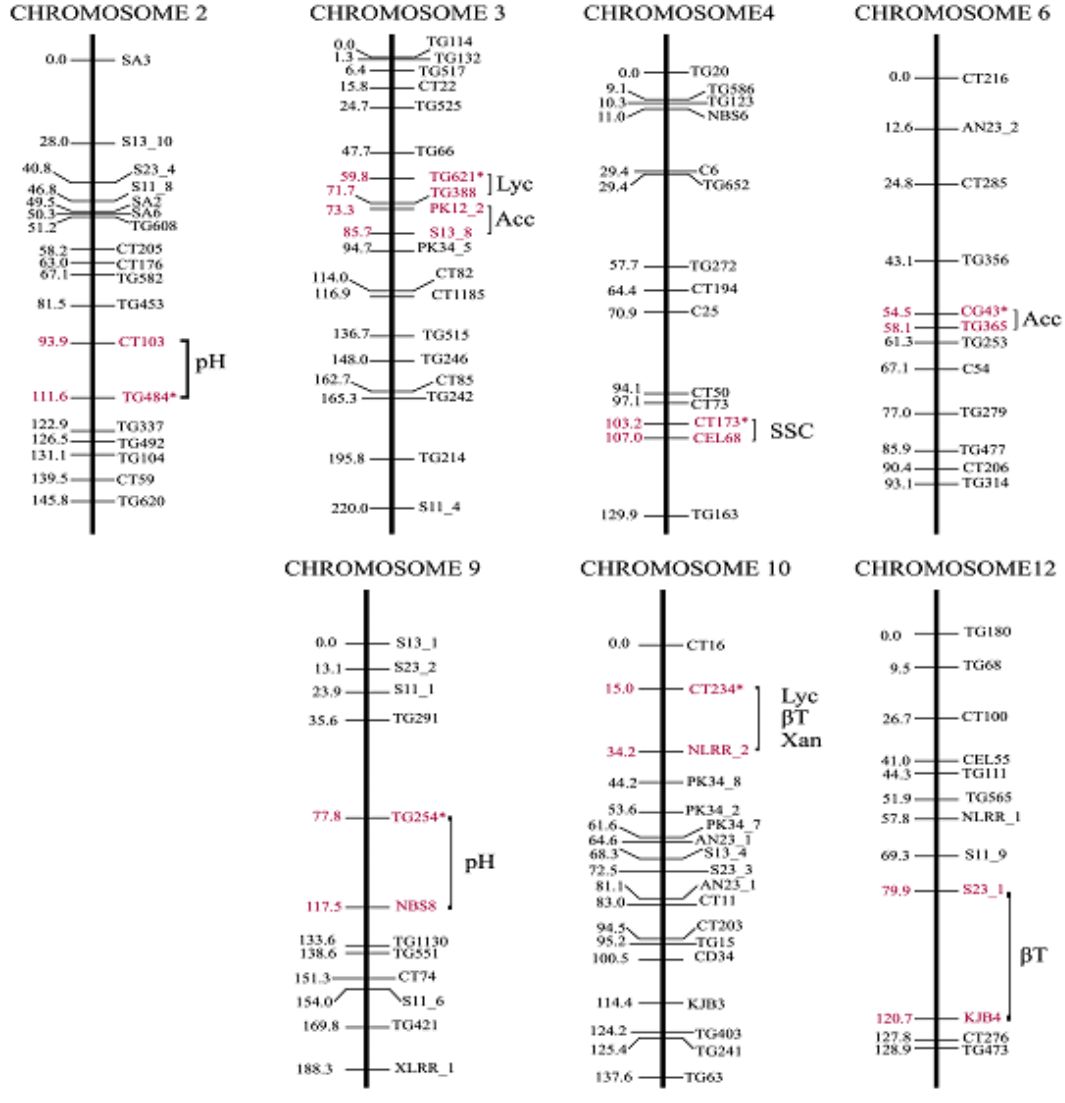

Figure 1. Molecular genetic linkage map of tomato based on 121 RFLP and 67 RGA using a recombinant inbred line (RIL) population derived from *Lycopersicon esculentum* and *L. pimpinellifolium*. The positions of the QTLs are presented on the left-hand side of the linkage groups. Bars represent intervals associated with the QTLs.

188cM, respectively), whereas chromosome 6 had the smallest linkage group (93.1 cM), followed by chromosome 11(114.4 cM).

Table 3 shows the QTLs mapped for various traits, marker intervals of peak LOD, number of locations in each QTL, additive effect and variation explained (%)as well asthe peak LOD. Ten QTLs were detected for the six evaluated traits (Table 3 and Fig.1). They explained 6.4-20.7% of the individual phenotypic variation.

For pH, two QTLs on chromosomes 9 and 2 were identified. The QTL on chromosome 9 had a large effect, explaining 20.7% of thevariation, with an LOD score of 4.92, whereas the QTL onchromosome 2 accounted for only 8.5% of the variation, with an LOD score of 2.02. One QTL for acidity was mapped in interval of CG43-TG395 markers on chromosome 9 explaining 10.5% of the

Table 2. Simple correlation coefficients among traits from 116 RILs derived from *Lycopersicon esculentum* and *L. pimpinellifolium*.

	pH	Acidity	Soluble solids	Lycopene	Beta-carotene	Xanthophyll
pH	1					
Acidity	- 0.21*	1				
Soluble solids	0.23*	0.56**	1			
Lycopene	0.10^{ns}	0.23*	0.30**	1		
Beta-carotene	0.05^{ns}	0.24*	0.30**	0.97**	1	
Xanthophyll	0.09^{ns}	0.23*	0.29**	0.96**	0.95**	1

*** and **: Significant at the 5% and 1% of probability levels.**
ns: Not significant.

Table 3. QTLs detected for chemical traits based on composite interval mapping in 116 RILs derived from *Lycopersicon esculentum* and *L. pimpinellifolium*. The most closely associated marker locus is indicated by *.

Trait	Interval	Chromosome	Interval length (cM)	LOD	Additive effect	Variation (%)
pH	TG254*-NBs8	9	1.98	4.92	0.04	20.7
	CT103-TG484*	2	3.69	2.02	-0.03	8.5
Acidity	CG43*-TG395	6	0.02	3.34	-0.38	10.5
	PK12-2-S13-8*	3	0.34	2.457	-0.34	10.0
Soluble solids	CT173*-CEL68	4	0.03	2.114	-0.31	7.0
Lycopene	CT234*-$NLRR_2$	10	1.22	2.37	-4.53	8.3
	TG621*-TG388	3	0.04	2.22	-0.02	7.0
Beta-carotene	CT234-NLRR-2*	10	3.22	2.60	-0.03	10.3
	S23*-1-KJB4	12	0.03	2.06	0.02	6.4
Xanthophyll	CT234-NLRR-2*	10	3.22	2.95	-0.04	13.7

variance with an LOD score of 3.34. The QTL allele in the locus inherited fromthe parent LA722 increased acidity by 0.38.

One QTL was detected forsoluble solids content which accounted for 7%of the total variation, with an LOD score of 2.114. The allele from the parent LA722 acted to increase the soluble solids content with an additive effect of 0.31.

The QTL on chromosome 10 explained 8.3% of the phenotypic variation for lycopene concentration and had an LOD score of 2.37. At this locus, the LA722 allele increased lycopene concentration by 4.53.

A total of three QTLs on chromosomes 3,10, and 12 influenced beta-carotene concentration. A QTL mapped to the CT234-NLRR-2 region of chromosome 10 had the largest effect, explaining 10% of the phenotypic variance, with an LOD score of 2.6. The QTLs on chromosomes 3 and 12 mapped to the TG621-TG388 and S23-1-KJB4 regions, respectively, and explained 7 and 6.4% of the phenotypic variance, respectively. The QTLs on chromosomes 3 and 10 showed an increase inbeta-caroteneassociated with LA722 alleles, whereas the QTL on chromosome 12 with an LOD score of 2.60 increased beta-caroteneby 0.02.

In addition, one QTL for xanthophyll concentration was found on chromosome 10 in the CT234-NLRR-2 region. This QTL explained 13.7% of the total variation, with an LOD score of 2.95. The allele from the parent LA722 at this locus increased xanthophyll concentration by 0.04.

DISCUSSION

To understand the genetic control of chemical traits in tomato, 6 traits in 116 RILs derived from a cross between *L. esculentum* and *L. pimpinellifolium* were evaluatedin this study. Many *L. pimpinellifolium* accessions are known to the tomato industry (Grandillo and Tanksley, 1996; Chen *et al.*, 1999). Accession LA722 used in this study exhibits high valuesfor soluble solids content, fruit acidity, fruit lycopene concentration, fruit beta-carotene concentration, and fruit xanthophyll concentration, but a low value of pH, which is important in tomato improvement.

High soluble solids content increases processed product yield (Rick, 1974) and the overall flavor of the fruit in fresh-market tomatoes (Stevens and Rick, 1986). In addition, acidity and pH are important criteria for tomato eating quality. Traits related to fruit color, such as lycopene, beta-carotene, and xanthophyll, are important for nutritional quality (Chen *et al.,* 1999) .Results of epidemiological studies have shown that tomatoes and tomato products may help protect against various human diseases, such as cancer (especially prostate cancer) and cardiovascular disease (Arab *et al*., 2000; Barber and Barber, 2002; Rao and Agarwal, 1999).

Parent values were significantly different for all six traits, and the parent LA722 showed higher values for most traits (data not shown). Transgression, observed for all traits except PH, could be explained by the presence of complementary QTL alleles in the two parents. As in a previous study (Saliba-Colombani *et al.,* 2001),

lycopene and beta-carotene were found to be positively correlated. Moreover, a positive moderate correlation between soluble solids content and fruit acidity was found in our study, which is consistent with the results of Saliba-Colombani *et al.* (2001).

The total map length used in this study is 1806 cM, with a mean density of one marker per 9.6 cM, which is lower than that of a previous map (Chen *et al.,*1999) constructed using 151RFLPs with a mean density of one marker per 7.9cM.

In this study, ten QTLs were detected for evaluated traits. The QTL for pH on chromosome 9 explained a large part of the phenotypic variation and thus may be considered a major gene. Meanwhile, the lycopene, beta-carotene, and xanthophyll contents explainedonly a small part of the phenotypic variation. For most QTLs (on different chromosomes), LA722 alleles contributed to increased acidity, soluble solids, lycopene, beta-carotene, and xanthophyll content, consistent with parental phenotypes. These results indicated the potential for improving cultivated tomatoes by marker-assisted introgression of QTLs from related wild species.

Beta-carotene

The co-localization of QTLs for beta-carotenewith QTLs for lycopene and xanthophyll could be due to a physiological relationship. These co-locations may also reflect the linkage between two genes or the pleiotropic effect of one gene. However, linkage of genes cannot be ruled out as a possible cause unless additional fine-resolution mapping or molecular cloning of QTLs isperformed (Chen *et al.,* 1999; Frary *et al.,* 2004).

Our results demonstrate that the *L. pimpinellifolium* accession, LA722, contains alleles capable of improving tomato fruit carotenoids, soluble solids content, and acidity, which are economically important in processing tomatoes. These QTLs may be useful in marker-assisted breeding of large-fruited, fresh-market tomatoes by introgression of QTLs from wild species.

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