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Mapping of quantitative trait loci for antioxidant molecules in tomato fruit: Carotenoids, vitamins C and E, glutathione and phenolic acids

Nergiz Gürbüz Çolak[a], Neslihan Tek Eken[a], Mehmet Ülger[b], Anne Frary[a], Sami Doğanlar[a,*]

[a] Department of Molecular Biology and Genetics, Faculty of Science, Izmir Institute of Technology, Izmir 35430, Turkey
[b] MULTI Tarım Seed Company, Antalya 07112, Turkey

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ABSTRACT

The nutritional value of a crop lies not only in its protein, lipid, and sugar content but also involves compounds such as the antioxidants lycopene, β-carotene and vitamin C. In the present study, wild tomato *Solanum pimpinellifolium* LA 1589 was assessed for its potential to improve antioxidant content. This wild species was found to be a good source of alleles for increasing β-carotene, lycopene, vitamin C and vitamin E contents in cultivated tomato. Characterization of an LA 1589 interspecific inbred backcross line (IBL) mapping population revealed many individuals with transgressive segregation for the antioxidants confirming the usefulness of this wild species for breeding of these traits. Molecular markers were used to identify QTLs for the metabolites in the IBL population. In total, 64 QTLs were identified for the antioxidants and their locations were compared to the map positions of previously identified QTLs for confirmation. Four (57 %) of the carotenoid QTLs, four (36 %) of the vitamin QTLs, and 11 (25 %) of the phenolic acid QTLs were supported by previous studies. Furthermore, several potential candidate genes were identified for vitamins C and E and phenolic acids loci. These candidate genes might be used as markers in breeding programs to increase tomato's antioxidant content.

1. Introduction

Tomato (*Solanum lycopersicum*) is an economically important member of the *Solanaceae* family which contains more than 2700 species including potato, eggplant, pepper, petunia, physalis, and tobacco [1]. The largest genus of the *Solanaceae* family is *Solanum* which contains approximately 1500 species. *Solanum* plants can be grown on all temperate and tropical continents and show a wide range of morphological and ecological diversity. The genus contains species producing medicinal compounds and economically important crops [2]. Tomato is part of the daily diet in most of the world and is widely used both fresh and processed in products such as paste, soup, juice, powder and concentrate. Tomato is a highly nutritive fruit, and its nutritional value is not considered to be confined to its protein, lipid, and sugar content but also includes compounds which are important to human health such as antioxidants [3].

Antioxidants are molecules that are capable of neutralizing the detrimental effects of reactive oxygen species (ROS) formed as a result of metabolic processes [4]. ROS have free radical groups which make them highly reactive. They disrupt the chemical bonds of nearby molecules. If ROS are not neutralized, they can cause oxidative damage to proteins, lipids and even DNA. Such damage to biological molecules can result in various ailments such as cancer, neurodegenerative or cardiovascular diseases [5–7]. Moreover, because the liver is the recycling center for ROS, liver diseases are thought to be initiated primarily due to high ROS concentrations [8]. Thus, ROS must be neutralized or recycled immediately by antioxidant molecules after they are produced [4]. Although humans can synthesize antioxidant enzymes, important antioxidant molecules such as vitamin C, vitamin E and β-carotene cannot be synthesized in the body, therefore antioxidant intake is required in the daily diet [9].

Glutathione, vitamins C and E, carotenoids and phenolic acids are antioxidant molecules found in fruits and vegetables [10–12]. Tomato is a good source of antioxidant molecules, especially lycopene, a type of carotenoid. Regular consumption of tomato can reduce the risk of chronic and cardiovascular disease, different types of cancer, and inflammation due to the interaction of tomato's phytochemicals with metabolic pathways which are related to the body's inflammatory response and oxidative stress [11].

Understanding the genetic basis of antioxidant synthesis in tomato provides powerful tools for breeders to develop new cultivars rich in antioxidant molecules. Moreover, antioxidant-rich varieties can be used as a source of phytochemicals for dietary supplement formulations and drugs. Antioxidant-based drugs, such as deprenyl and tocopherol, have

* Corresponding author.
E-mail address: samidoganlar@iyte.edu.tr (S. Doğanlar).

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been used in clinical trials for therapy of Parkinson's disease [10]. Therefore, antioxidant molecules are important for both their nutritional benefits and for the prevention and treatment of diseases.

Introgression of wild alleles into cultivars is an important breeding strategy to improve antioxidant traits, because wild relatives are rich in antioxidant molecules [13]. For example, *S. pimpinellifolium* is rich in lycopene [14–19], vitamin C [13,15,16,19], phenolic acids [16] and has higher antioxidant capacity than cultivated tomato [13,16]. *S. pennellii* introgressions improved the content of tomato antioxidant molecules including vitamin E [20,21], vitamin C [12,22–25], carotenoids [22], glutathione [26], flavonoids, phenolic acids [12,24,26–30], and antioxidant activity [24,30]. In addition, *S. chmielewskii*, *S. habrochaites* and *S. peruvianum* were shown to have higher phenolic acid content than cultivated tomato [13,31,32]. Antioxidant capacity of *S. habrochaites* [13,32] and *S. peruvianum* [13] also exceeded that of cultivated tomato.

In the present work, quantitative trait loci (QTLs) for antioxidant molecules were identified using an inbred backcross line (IBL) population derived from a cross between the wild tomato species *S. pimpinellifolium* and a freshmarket tomato (*S. lycopersicum*) cultivar. To this end, we performed targeted metabolic profiling of phenolic acids, carotenoids, glutathione, vitamin C, and vitamin E and used whole-genome sequencing data from our previous work [33] to identify single nucleotide polymorphism (SNP) markers linked to the antioxidant traits. Marker positions were then used to identify possible candidate genes.

2. Materials and methods

2.1. Chemicals and instrumentation

Standard molecules used in HPLC analysis were analytical grade or min. 99 % purity and purchased from Applichem and Sigma. All solvents used in HPLC analysis and solution/buffer preparation were HPLC grade and purchased from VWR Chemicals. Quantification of metabolites was done with a Shimadzu LC-20 AT model HPLC-PDA/FLD using HPLC columns by GL Sciences (RP C18, 5 µm – 25 × 4.6 mm) and Shimpack (RP C18, 3 µm – 10 × 2.1 mm).

2.2. Plant material

An interspecific IBL (inbred backcross line) population derived from the cross *S. lycopersicum* cv. Tueza x *S. pimpinellifolium* (LA 1589) was used as plant material in the study. Tueza is a cultivated fresh market tomato line with large (150–160 g), red, slightly flattened round fruits. LA 1589 is a wild tomato accession with small, red, round fruits. The IBL population and parents were grown in the greenhouse in Antalya, Turkey. Ten plants per genotype were grown in double rows with 140 and 30 cm between wide and narrow rows, respectively. Plants were spaced at 40 cm intervals within rows. For basal fertilization, 500 kg 15:15:15 (N:P:K) fertilizer and 50 t of composted manure were applied per ha. Drip irrigation was used with fertigation (1.4 dS m^{-1} EC value) at each irrigation using 1-2-1 fertilizer until first fruit set, 2-1-1 fertilizer until first fruit ripening and 1-1-2 fertilizer after first fruit ripening.

2.3. Targeted metabolic profiling

Ripe fruits from 10 plants of each of the population's 94 individuals and two parents were bulked and diced. Samples (100 g) were then lyophilized. A fine powder was obtained from dried samples with a knife mill grinder. A total of 1 g of each dried tomato sample was extracted in 5 ml hexane:dichloromethane (1:1, v/v) on an orbital shaker at 400 rpm at 18 °C overnight. Samples were centrifuged at 4 °C, 4000 rpm for 20 min. Supernatants were saved and pellets were subjected to a second extraction with the same solvent overnight. After centrifugation of the samples, the apolar extract supernatants were combined, aliquoted and stored at −80 °C until analysis. The pellets were then subjected to another extraction with chloroform:methanol:water (1:3:1, v/v/v) on an orbital shaker at 400 rpm at 18 °C overnight. The same procedure was followed as the hexane:dichloromethane extraction described above to obtain the polar extracts which were also stored at −80 °C until analysis. Two technical replicates were performed for each sample for each analysis. All results are expressed as mg/100 g dry weight (DW).

Carotenoids were analyzed with two different methods. Apolar extracts were analyzed with an isocratic method of HPLC-PDA which was combined and modified from the methods described in previous reports [34,35]. Lycopene and β-carotene were analyzed on a reverse phase (RP C18, 5 µm – 25 × 4.6 mm) column at 30 °C using methanol:ethyl acetate:acetonitrile (50:40:10, v:v:v, with 0.05 % triethylamine added to ethyl acetate and acetonitrile) as the mobile phase with a 1.5 ml/min flow rate. Sample injection was 20 µl and standard solutions of lycopene and β-carotene were prepared in methanol:acetone (1:1, v:v) and dichloromethane, respectively. Detection was done at 450 nm and 469 nm with a PDA detector. Lutein and zeaxanthin were analyzed from the apolar extracts on a reverse phase (RP C18, 5 µm – 25 × 4.6 mm) column at 30 °C using acetonitrile:methanol (10:90, v:v with 0.05 % triethylamine added to acetonitrile) as the mobile phase with a 1 ml/min flow rate. Sample injection was 20 µl and standard solutions of lutein and zeaxanthin were prepared in dichloromethane containing 0.01 % BHT. Detection was done at 475 nm with PDA detector.

Vitamin C was analyzed in polar extracts with an isocratic method of HPLC-PDA which was combined and modified from the methods previously described by Li and Chen [36,37]. Vitamin C was analyzed on a reverse phase (RP C18, 5 µm – 25 × 4.6 mm) column at 40 °C using methanol:potassium dihydrogenphosphate buffer (0.1 M KH_2PO_4, pH 7) (10:90, v:v) as the mobile phase with a 1 ml/min flow rate. Sample injection was 20 µl and standard solutions were prepared in ultrapure water. Detection was done at 265 nm with a PDA detector.

Vitamin E was analyzed in apolar extracts with an isocratic method of HPLC-FLD which combined and modified the methods of Bakre et al. [38] and Turner et al. [39]. Vitamin E was analyzed on a reverse phase (RP C18, 5 µm – 25 × 4.6 mm) column at 40 °C using acetonitrile:methanol (75:25, v:v) as the mobile phase with a 1.5 ml/min flow rate. Sample injection was 20 µl and standard solutions were prepared in acetonitrile:methanol (80:20, v:v). Vitamin E was detected with a fluorescence detector at 300 nm excitation and 360 nm emission.

Reduced and oxidized glutathione were analyzed from polar extracts with an isocratic method of HPLC-PDA which was modified from the method previously described by Khan et al. [40]. Glutathiones were analyzed on a reverse phase (RP C18, 3 µm – 10 × 2.1 mm) column at 35 °C using 0.05 % trifluoroacetic $acid_{(aq)}$:methanol (97:3, v:v) as the mobile phase with a 0.2 ml/min flow rate. Sample injection was 20 µl and standard solutions were prepared in 0.05 % trifluoroacetic $acid_{(aq)}$. Detection was at 208 nm with a PDA detector.

Phenolic acids were analyzed from polar extracts with a gradient method (Table S1) of HPLC- PDA which was modified from the method previously described by Gomez-Alonso et al. [41]. Samples were run on a reverse phase (RP C18, 5 µm – 25 × 4.6 mm) column at 35 °C using (A) ammonium dihydrogen phosphate buffer ($NH_4H_2PO_4$, 50 mm, pH 2.6), (B) 20 % mobile phase A and 80 % acetonitrile, and (C) 200 mM phosphoric acid as the mobile phase with a 1 ml/min flow rate. Sample injection was 20 µl and standard solutions were prepared in methanol. Detection was done at 280, 320, 360, 520 nm with a PDA detector.

2.4. Genomic data and QTL mapping

Genomic data were obtained as described in our previous study [33]. In total, 3125 genome-wide SNPs obtained by genotyping by sequencing (GBS) were used in QTL mapping of the antioxidant traits. Log2 transformation was performed for the metabolic data. QGene version 4.0 [42] was used for QTL analysis. The CIM (Composite

Interval Mapping) QTL analysis method combines both interval mapping and multiple regression analysis and was performed with automatic forward cofactor selection and a scan interval of 0.2 Mb. A LOD threshold ≥ 3.0 was used. QTLs identified in our study were compared to previously mapped loci to determine the reliability of the QTLs. The map locations of the previously mapped loci's nearest markers were compared and an overlap was assumed if the markers were within 4 Mb. Candidate genes around the identified QTLs associated with each trait were identified using the SolyCyc Biochemical Pathways tools on SGN (www.solgenomics.net). The full gene list in the QTL region plus the 1 Mb region on both sides was searched using the reference genome. Candidate genes were retained based on the possible biochemical pathways/reactions related to the identified QTL.

3. Results

3.1. Metabolic variation for antioxidant molecules

A total of 94 IBL individuals and parental accessions (cultivated parent *S. lycopersicum* cv. Tueza and wild parent *S. pimpinellifolium* acc. LA 1589) were characterized for four carotenoids including lycopene, β-carotene, lutein and zeaxanthin; two vitamins including vitamins C and E; two forms of glutathione, reduced and oxidized; and 31 phenolic compounds including flavanols, flavones, flavan-3-ols, hydroxybenzoic acids, hydroxycinnamic acids and anthocyanins. Overall, the wild parent's averages exceeded those of the cultivated parent for two of the four carotenoids, vitamins C and E, and nine of the 19 (47 %) phenolic compounds.

3.1.1. Carotenoids

The most abundant carotenoid in the parents as well as the IBL population was lycopene (Table 1). Both the parents and the population had high lycopene and β-carotene and low lutein and zeaxanthin content. The wild parent had 1.6 fold higher lycopene content than the cultivated parent. In contrast, the cultivated parent had 1.6 fold higher β-carotene content than the wild one. Average lycopene content of the IBLs was slightly higher than that of cultivated parent Tueza. The lycopene content of all individuals in the IBL population was much lower than wild parent LA 1589. The IBL population means were intermediate between the two parents for β-carotene and lutein; however, average zeaxanthin content in the IBLs was higher than both parents. In general, carotenoids showed moderate variation within the population with the exception of lycopene which had a low CV, 6.5 %. All traits except lycopene showed transgressive segregation in which some individuals in the mapping population exceeded the parental values for β-carotene, lutein and zeaxanthin. Lycopene tended to accumulate at high concentrations in many individuals of the population. The rest of the carotenoids displayed more normal continuous variation in the population (Supplementary material Fig. S1).

Table 1
Carotenoid, vitamins C and E, and glutathione content measured in the IBL population and parents: *S. lycopersicum* cv. Tueza and *S. pimpinellifolium* acc. LA 1589. Quantities of metabolites are given as mg 100 g^{-1} DW.

	Parents		IBL Population		
	Tueza (Mean)	LA 1589 (Mean)	Mean	Range	CV%
Carotenoids					
Lycopene	16141.58	26733.95	16919.46	11291.00-18518.74	6.5
β-Carotene	56.62	36.06	45.98	3.86-88.52	38.8
Lutein	3.25	5.06	3.83	0.04-7.95	32.4
Zeaxanthin	3.26	2.78	3.60	0.47-9.83	45.8
Vitamins					
Vitamin C	19.11	20.17	17.84	0-41.50	50.8
Vitamin E	3.61	20.28	20.40	0-57.76	80.9
Glutathione					
Reduced Glutathione	17.75	10.79	52.17	7.57-322.38	113.4
Oxidized Glutathione	5.18	0.09	56.14	0-510.60	186.5

3.1.2. Vitamins

The parents had nearly the same amount of vitamin C, approximately 20 mg 100 g^{-1} DW (Table 1), which was more than the mean vitamin C content for the population. Despite these similarities in mean values, vitamin C content of the IBLs ranged widely with a CV of 50.8 % and values up to 41.50 mg 100 g^{-1} DW. Although the distribution of vitamin C content in the population skewed toward the lower values, considerable transgressive variation for the trait was observed (supplementary material Fig. S2). The wild parent had much higher vitamin E content than the cultivated one: 20.28 vs 3.61 mg 100 g^{-1} DW, a 5.6 fold difference (Table 1). The mean vitamin E content of the population was similar to the wild parent. Vitamin E displayed high variation in the population with a CV of 80.9 % and some individuals had vitamin E content that was 30 fold higher than the tomato cultivar and 6 fold greater than the wild species indicating significant transgressive segregation (Supplementary material Fig. S2).

3.1.3. Glutathione

The cultivated parent had higher amounts of reduced and oxidized glutathione than the wild parent (Table 1). The mean values for both traits in the population were much higher than even the cultivated parent, Tueza. The IBLs averaged three fold more reduced glutathione and 10.9 fold more oxidized glutathione than Tueza. Although transgressive segregation occurred in the population which had high variation, glutathiones did not display continuous distribution (Supplementary material Fig. S3). Instead most individuals were similar to parental values and did not have extreme glutathione contents.

3.1.4. Phenolic acids

Nineteen different phenolic acids were identified in the plant material (Table 2). Syringetin, kaempferol, isorhamnetin, naringenin, taxifolin, cyanidine, delphinidine, pelargonidin, peonidin, cafteric acid, resveratrol and pterostilbene were not detected in the parents or in the IBL population. In general, when individual compounds were totaled within phenolic acid subclasses, the wild parent had higher amounts than the cultivated parent. The only exceptions were hydroxybenzoic acids, which were present in a much higher amount in the cultivated parent, and anthocyanins which were found in equal amounts in both parents. At the level of individual phenolic acids, the wild parent had higher levels for approximately half of the compounds. Each parent had a distinct phenolic acids profile. Compared to the wild parent, the cultivated parent Tueza was very rich in gallic acid (30 fold more), chlorogenic acid (23 fold more), vanillic acid (six fold more) and hydroxyl benzoic acid (five fold more). In contrast, LA 1589 was comparatively rich in ferulic acid (15 fold more), coumaric acid (nine fold more), syringic acid (five fold more) and myricetin (four fold more). The IBL population was found to contain higher average amounts of all classes of phenolic acids than the parents, with the exception of hydroxycinnamic acids (Table 2). All traits had high variation in the population with CVs ranging from 93.6 % to 287.6 %. Phenolic acids did not display normal continuous distribution and tended to accumulate in low amounts in many individuals of the population (Supplementary material Fig. S4).

3.2. QTL mapping and candidate genes

Based on a LOD threshold of 3.0, 64 QTLs including seven for carotenoids, 11 for vitamins, three for glutathiones, and 43 for phenolic acids were identified on all 12 tomato chromosomes for 22 of the 27 quantified compounds (Tables S2–S5). The QTLs were found to colocalize many times on chromosomes 1, 3, 4, 7, 8, 9, and 10 (Table S6)

Table 2
Phenolic acid content measured in the IBL population and parents: *S. lycopersicum* cv. Tueza and *S. pimpinellifolium* acc. LA 1589. Quantities of metabolites are given as mg 100 g^{-1} DW.

	Parents		IBL Population		
	Tueza (Mean)	LA 1589 (Mean)	Mean	Range	CV%
Flavanols					
Quercetin	1.88	1.67	1.025	0 – 7.09	116.0
Myricetin	10.00	40.00	3.06	0 – 17.73	132.7
Syringic acid	3.96	20.40	89.46	0 – 1127.78	257.8
Total	15.84	61.67	93.54		
Flavones					
Chrysin	0.42	2.07	80.12	0 - 888.34	240.9
Apigenin	2.18	1.24	13.04	0.26 - 72.10	126.7
Luteolin	0.62	0.20	0.72	0 – 3.89	120.6
Total	3.22	3.51	93.88		
Flavan-3-ols					
Catechin	0.37	0.59	26.53	0 - 249.50	191.3
Epicatechin	0.24	5.89	2.45	0 - 44.02	287.6
Epigallocatechin	1.92	2.19	2.61	0 - 24.74	120.6
Total	2.53	8.67	31.59		
Hydroxybenzoic acids					
Gallic acid	31.46	1.06	5.07	0 - 26.33	146.1
Vanillic acid	12.8	2.05	61.12	0 – 680.02	213.9
Hydroxy benzoic acid	6.60	1.37	46.80	0 - 275.41	163.4
Total	50.86	4.48	112.99		
Hydroxycinnamic acids					
Cinnamic acid	0.06	0.11	0.98	0 - 8.20	171.8
Coumaric acid	0.24	2.13	1.87	0 - 21.80	221.2
Ferulic acid	2.08	31.00	3.29	0 - 28.16	192.1
Caffeic acid	9.54	3.32	3.99	0.1 – 38.42	175.7
Sinapic acid	1.47	1.29	1.60	0 – 9.36	128.7
Chlorogenic acid	19.20	0.83	0.53	0 – 2.66	93.6
Toal	32.59	36.68	12.26		
Anthocyanins					
Malvidin	0.76	0.75	3.48	0 - 40.56	179.2

and many traits were significantly correlated ($P < 0.05$, Table S7). Candidate genes were identified for vitamin C (two candidate genes) and phenolic acids (16 candidate genes) (Table 3).

3.2.1. Carotenoids

A total of seven QTLs were identified for carotenoids (Table S2). Three QTLs were identified on chromosomes 8 and 9 for lycopene with percentages of phenotypic variation explained (PVE) varying between 15 % and 35 %. The QTL (*lyc8.1*) with the greatest effect was found on chromosome 8, and, at this locus, wild parent alleles contributed to increased lycopene content. Increased lycopene content for the two loci on chromosome 9 was associated with cultivated parent alleles. Three QTLs were identified for β-carotene on chromosomes 8, 10 and 11. The major QTL (*βcrn8.1*, PVE = 34 %) on chromosome 8 had increased β-carotene content associated with wild parent alleles. This QTL was colocalized with the lycopene locus on chromosome 8. For the minor QTLs on chromosomes 10 and 11, cultivated parent alleles were associated with increased and decreased β-carotene content, respectively. Only one minor QTL was identified for zeaxanthin on chromosome 4 for which the increased content came from the wild parent's allele. No QTL was detected for lutein.

3.2.2. Vitamins

Eleven QTLs were identified for vitamin content (Table S3). Six QTLs were identified for vitamin C on chromosomes 6, 7, 8, 10 and 12. Two of the QTLs, on chromosome 6 and 7, had a moderate effect ($20 < PVE < 30$) and increased vitamin C content was associated with *S. pimpinellifolium* alleles. The rest of the loci were minor QTLs ($PVE < 20$). Both wild and cultivated alleles were responsible for increased content at these loci. For vitamin E content, five QTLs were identified on chromosomes 5, 9 and 10. The QTLs on chromosome 5 had minor effects. One of the QTLs on chromosome 9 was minor while one, *vite9.2,* was major with a PVE of 55 %. In addition, there was a major QTL on chromosome 10 which had a 51 % magnitude of effect. Alleles from the cultivated parent were associated with higher vitamin E content for the loci on chromosome 5 while wild parent alleles had this effect on chromosomes 9 and 10.

3.2.3. Glutathione

Three QTLs were identified for glutathione (Table S4). One of the two QTLs on chromosome 1 had a moderate effect while the other had a minor effect. The locus on chromosome 4 was also a minor QTL. All identified loci for increased glutathione were associated with alleles from the cultivated parent.

3.2.4. Phenolic acids

A total of 43 QTLs were identified for phenolic acids. The phenolic acids fell into several categories: flavanols, flavones, flavan-3-ols, hydroxybenzoic acids, hydroxycinnamic acids and anthocyanins (Table S5). Seven QTLs were identified for flavanols, including three QTLs for quercetin with minor effect, and four QTLs for syringic acid. One of the QTLs on chromosome 6 for syringic acid (*sya6.1*) was considered as major with a PVE of 35 % while the QTL on chromosome 2 had a moderate effect. The other two QTLs were minor. Alleles of the cultivated parent were responsible for increased quercetin content for two of the three loci. Both parents were equally associated with increased syringic acid content.

Six QTLs were identified for flavones, including two for chrysin and four for apigenin. For chrysin, the QTL on chromosome 1 had a minor effect. Chromosome 8 had a long locus for chrysin content with a moderate effect. The alleles of the cultivated parent were associated with increased chrysin content for both loci. Two of the QTLs for apigenin on chromosome 1 and 11 had a moderate effect, with wild alleles controlling increased apigenin content. The other two QTLs on chromosome 2 and 9 were considered as minor QTLs, and alleles of both parents were equally responsible for increases in the trait.

Nine QTLs were identified for flavan-3-ols, including three for each identified compound. Two of the three QTLs for catechin were considered as minor, whereas the QTL on chromosome 7 had a moderate effect. Increased catechin content for two of the QTLs was associated with cultivated parent alleles. All three QTLs for epicatechin were minor QTLs. Wild parent alleles for two of the identified QTLs were associated with increased epicatechin content. A major QTL (PVE 54 %) for epigallocatechin was detected on chromosome 1. The other identified QTLs on chromosomes 2 and 4 had moderate effects. Increased epigallocatechin content for two of the QTLs, including the major locus, was associated with cultivated parent allelic effects.

Six QTLs were detected for hydroxybenzoic acids, including a long QTL for gallic acid, three for vanillic acid and two for hydroxybenzoic acid. All these QTLs were minor loci. Increased gallic acid content was only associated with alleles from the cultivated parent. Increased vanillic acid content was associated with the wild parent allele at one locus. Increased hydroxybenzoic acid content was equally associated with alleles from both parents.

A total of 15 QTLs were identified for hydroxycinnamic acids, including one for cinnamic acid, three for coumaric acid, two for ferulic acid, three for caffeic acid, four for sinapic acid, and two for chlorogenic acid. All of the QTLs for hydroxycinnamic acids were minor QTL with the exception of the locus for chlorogenic acid on chromosome 6 which had a moderate effect. All of the loci responsible for increased cinnamic acid and ferulic acid content and most of those for coumaric acid were associated with alleles from the cultivated parent, while increased caffeic acid, sinapic acid, and chlorogenic acid content were associated with alleles from the wild parent.

No QTLs were detected for myricetin, lutein, and the anthocyanin, malvidin.

Table 3
Candidate genes for the antioxidant QTLs identified in the study.

Trait	QTL	Solgenomics ID	Position (Mb)	Mapman Annotation	Solgenomics annotation
Vitamin C	*vitc10.2*	SolyC10g083890.1*	63.6	TCA / org. transformation.carbonic anhydrases	Carbonic anhydrase family protein (AHRD V1 ***- D7KHY6_ARALY); contains Interpro domain(s) IPR018340 Carbonic anhydrase, CAH1-like
	vitc8.1	SolyC08g068800.2	57.9	Redox.ascorbate and glutathione.glutathione	Glutathione peroxidase (AHRD V1 ***- B0FYJ0_9ROSI); contains Interpro domain(s) IPR012335 Thioredoxin fold
Vitamin E[a]	*vite5.2*	none	nd	none	4-hydroxyphenylpyruvate dioxygenase
	vite9.2	none	nd	none	dimethyl-phytylquinol methyl transferase, phytol kinase, phospholipid transporter
Chrysin	*cry8.1*	SolyC08g014360.1	4.2	Secondary metabolism.phenylpropanoids.lignin biosynthesis.CAD	Cinnamyl alcohol dehydrogenase-like protein (AHRD V1 **** A9PHZ1_POPTR); contains Interpro domain(s) IPR002085 Alcohol dehydrogenase superfamily, zinc-containing
	cry8.1	SolyC08g014490.1	4.6	Secondary metabolism.phenylpropanoids	Hydroxycinnamoyl CoA quinate transferase (AHRD V1 **-* Q70G33_TOBAC); contains Interpro domain(s) IPR003480 Transferas
Apigenin	*apn9.1*	SolyC09g066310.2*	64.7	Hormone metabolism.ethylene.synthesis-degradation	Flavonol synthase/flavanone 3-hydroxylase (AHRD V1 **-* B6SHP9_MAIZE); contains Interpro domain(s) IPR005123 Oxoglutarate and iron-dependent oxygenase
Catechin	*ctn2.1*	SolyC02g089770.2*	51.4	Secondary metabolism.flavonoids.dihydroflavonols	Dihydroflavonol-4-reductase (AHRD V1 ***- B6TK03_MAIZE); contains Interpro domain(s) IPR016040 NAD(P)-binding domain
Epicatechin	*ectn1.1*	SolyC01g087640.2*	82.5	Secondary metabolism.flavonoids.dihydroflavonols	Cinnamoyl CoA reductase-like protein (AHRD V1 ***- A9PFK4_POPTR); contains Interpro domain(s) IPR016040 NAD(P)-binding domain
	ectn11.1	SolyC11g044830.1*	32.2	Secondary metabolism.flavonoids.dihydroflavonols	Flavanone 3-hydroxylase (AHRD V1 **– D7LTS6_ARALY); contains Interpro domain(s) IPR005123 Oxoglutarate and iron-dependent oxygenase
	ectn11.1	SolyC11g044820.1	32.2	Secondary metabolism.flavonoids.anthocyanins	Flavonol synthase/flavanone 3-hydroxylase (AHRD V1 **– B6U6C1_MAIZE); contains Interpro domain(s) IPR005123 Oxoglutarate and iron-dependent oxygenase
Vanillic acid	*va6.1*	SolyC06g043130.1*	29.7	Secondary metabolism.flavonoids.chalcones	Chalcone synthase (AHRD V1 ***- B9P9S8_POPTR); contains Interpro domain(s) IPR001099 Chalcone/stilbene synthase, N-terminal
	va6.1	SolyC06g043120.1*	29.7	Secondary metabolism.flavonoids.chalcones	Chalcone synthase (AHRD V1 ***- B9P9S8_POPTR); contains Interpro domain(s) IPR001099 Chalcone/stilbene synthase, N-terminal
Cinnamic acid	*cna4.1*	SolyC04g076380.2	61.2	Secondary metabolism.phenylpropanoids	Cytochrome P450 NADPH-reductase (AHRD V1 **** B3RFK2_PETHY); contains Interpro domain(s) IPR015702 NADPH Cytochrome P450 Reductase
Sinapic acid	*sa3.1*	SolyC03g031470.2*	4.0	Secondary metabolism.flavonoids.dihydroflavonols	Dihydroflavonol 4-reductase (AHRD V1 **** B9 × 2I6_ROSHC); contains Interpro domain(s) IPR016040 NAD(P)-binding domain
	sa3.1	SolyC03g032220.2	4.7	Secondary metabolism.phenylpropanoids.lignin biosynthesis.CCoAOMT	Caffeoyl-CoA O-methyltransferase (AHRD V1 **** A2PZD5_IPONI); contains Interpro domain(s) IPR002935 O-methyltransferase, family 3
Chlorogenic acid	*chla12.1*	SolyC12g005350.1*	0.2	Secondary metabolism.flavonoids.dihydroflavonols	Dihydroflavonol-4-reductase (AHRD V1 ***- B6TUD3_MAIZE); contains Interpro domain(s) IPR016040 NAD(P)-binding domain
		Solyc12g005640.1	0.3	RNA.regulation of transcription.MYB domain transcription factor family	Myb-related transcription factor (AHRD V1 *— B5RHV2_MUSBA); contains Interpro domain(s) IPR015495 Myb transcription factor
		Solyc12g005800.1	0.4	not assigned.unknown	MYB transcription factor MYB73 (AHRD V1 ***- Q0PJH5_SOYBN); contains Interpro domain(s) IPR015495 Myb transcription factor
		Solyc12g005890.1	0.5	RNA.regulation of transcription.MYB domain transcription factor family	Myb-related transcription factor (AHRD V1 *-*- B7UCQ7_PANGI); contains Interpro domain(s) IPR015495 Myb transcription factor

*Mapped genes.
[a]Genes mapped by Almeida et al. [20] but not found in genomic sequence at QTL positions.

3.3. Colocalized and correlated traits

Some of the antioxidant traits colocalized on seven of the 12 tomato chromosomes (Table S6). On chromosome 1 at 0.6 Mb, a ferulic acid QTL colocalized with chrysin and apigenin loci while at the 64.6 Mb position, a ferulic acid QTL colocalized with an epigallocatechin locus. QTLs for quercetin and vanillic acid colocalized at the 26–28 Mb position on chromosome 3. On chromosome 4, glutathione and hydroxybenzoic acid QTLs were both located at 0.5 Mb. Chromosome 4 also had colocalization of quercetin and epigallocatechin QTLs at 50.5 Mb, and zeaxanthin and cinnamic acid loci at 60.5 Mb. Catechin and vanillic acid QTLs mapped to the same region of chromosome 7. QTLs for lycopene and β-carotene mapped together at the 62 Mb position on chromosome 8. Chrysin and gallic acid QTLs colocalized twice on chromosome 8 at the 10 Mb and 36 Mb positions. A vitamin E locus mapped with lycopene at the 30.7 Mb position on chromosome 9. On chromosome 10, vitamin E colocalized with a vitamin C QTL at the 6 Mb position, and with a sinapic acid QTL at the 38 Mb position.

Significant positive correlations between the antioxidant traits were observed (Table S7). Oxidized glutathione and syringic acid were positively correlated (r^2 = 0.52). Other correlations were seen between phenolic acids, including weak and strong correlations. Positive strong correlations were seen between quercetin and epicatechin (r^2 = 0.64), myricetin and caffeic acid (r^2 = 0.64), myricetin and malvidin (r^2 = 0.88), and chlorogenic acid was strongly correlated with luteolin (r^2 = 0.52).

3.4. Candidate genes

A total of 18 candidate genes were identified for the antioxidant traits by examining genomic sequence in the QTL regions (Table 3). Two candidate genes were identified for vitamin C QTLs. The remaining candidate genes were found for phenolic acids QTLs. One of the two candidate gene (SolyC10g083890.1) for vitamin C was previously mapped (Sol Genomics network). Eight of the 16 candidate genes for phenolic acids QTLs were also mapped on the Sol Genomics network. These mapped genes were identified in the QTL regions for apigenin (SolyC09g066310.2), catechin (SolyC02g089770.2), epicatechin (SolyC01g087640.2, SolyC11g044830.1), vanillic acid (SolyC06g043130.1, SolyC06g043120.1), sinapic acid (SolyC03g031470.2), and chlorogenic acid (SolyC12g005350.1).

4. Discussion

Fruits and vegetables are good sources of antioxidants and minerals and, therefore, important for the human diet. Antioxidants can reduce the risk of developing an illness or prevent diseases like cancer or cardiovascular disease. Vegetable crop breeding strategies now include the selection and development of higher nutritional quality vegetables for their health benefits [43]. Tomato is an important contributor to human health due to its antioxidant content [44]. Vitamin C (ascorbic acid), vitamin E (α-tocopherol), carotenoids and phenolics are the main antioxidant molecules in tomato [45,46]. Glutathione is also a powerful antioxidant and is linked to vitamins C andvitamin E by oxidation-reduction reactions [47,48].

Unfortunately, studies on antioxidant capacity and antioxidant-related traits in tomato are limited and these have focused on vitamin C, vitamin E, carotenoids and total phenolic acids but not glutathione or individual phenolic acids. The existing studies on glutathione evaluated changes in glutathione content under stress conditions, especially salinity stress [49–53].

Characterization of the antioxidant traits in our study confirmed that the wild species *S. pimpinellifolium* acc. LA 1589 could be a useful parent for the development of antioxidant-rich cultivars because it contained more lycopene, lutein, vitamins C and E and some phenolic acids than *S. lycopersicum* cv. Tueza. However, the genetic potential of a given line is not revealed completely by its phenotype [54]. For example, in 1998 Bernacchi et al. [55] found that the green-fruited wild species *S. habrochaites* could be used to improve red fruit color in tomato. The limitation of phenotype to reveal genetic potential also holds true at the metabolic level as many individuals in our IBL population showed positive transgressive segregation for various antioxidant parameters. Transgressive segregation is a common occurrence in interspecific populations and involves “the assembly of ideal synergies across many genes and their alleles throughout the genome” [56]. This phenomenon may play a crucial role in the adaptation of natural populations especially when exposed to less than ideal environmental conditions [56]. Under such conditions, plants must defend themselves against various stress factors and antioxidant molecules are critical components of their defense strategy. Thus, it is not surprising that many instances of transgressive segregation were observed for these traits in our population. In this context, some of these transgressive lines can be used to breed for high vitamin E and glutathione content. Other lines had very high levels of syringic acid, chrysin, apigenin, catechin, vanillic acid, hydroxybenzoic acid, and malvidin. The benefits of these individual compounds for plant and human health is less obvious and should be studied further.

4.1. Metabolic variation

4.1.1. Carotenoids

Carotenoids make the highest contribution to tomato’s antioxidant capacity and it is primarily their intake that accounts for the nutritional importance of tomato consumption [46]. The main carotenoid in tomato is lycopene followed by β-carotene. Lutein and zeaxanthin are also found in tomato at low concentrations [11]. Lycopene was found to be the major carotenoid followed by β-carotene in both parents and the IBL population in agreement with Raiola’s study [11]. This result was expected because at the ripe stage, tomato lycopene levels increase mainly due to phytoene synthase-1 (Pys-1) enzyme activity which catalyzes the first step of carotenoid biosynthesis [57]. In our study, the wild parent *S. pimpinellifolium* had higher lycopene (267.3 mg g^{-1} vs 161.4 mg g^{-1}) and lutein levels than the cultivated parent while the cultivated parent Tueza had higher zeaxanthin and β-carotene (0.56 mg g^{-1} vs 0.36 mg g^{-1}) levels. Similar results were observed for lycopene and β-carotene in a previous study that examined *S. pimpinellifolium* acc. TO-937 and the cultivar Moneymaker [15]. Although *S. pimpinelifolium* was used in both studies, we found much higher content for both carotenoids in this wild species. This difference could arise from the use of different wild accessions (LA 1589 vs TO-937) and different environmental and growth conditions. The use of different cultivars also causes variation. For example, carotenoid content of commercial tomato Ailsa Craig (0.25 mg g^{-1} DW lycopene, 0.04 mg g^{-1} DW β-carotene) was lower than that of Tueza [58]. Although it is not possible to compare our results directly with additional studies because of the use of different units (dry weight vs. fresh weight), several other studies have found that lycopene levels in fresh fruit were higher in *S. pimpinellifolium* than in the cultivars/lines Ailsa Craig [17], NCEBR-1 [14] and 50 *S. lycopersicum* accessions [16], and S0805 [19]. In addition, the mean lycopene content of a *S. pimpinellifolium* LA 2093 BC_2 population was higher than the cultivated parent NCEBR-1, indicating that alleles for increased lycopene content were derived from the wild species [18]. Interestingly, despite the dramatic difference in lycopene content between our parental lines, relatively few of the IBL’s lycopene content exceeded that of the cultivated parent. This can be explained by the fact that each IBL’s genome is predominantly identical to that of the cultivated parent with only a few introgressions from the wild parent.

Lycopene is responsible for the red color of tomato fruit [59] while β-carotene plays a role in orange color [60]. The only red-fruited wild relative of tomato is *S. pimpinellifolium* [61] which, as described above, has a considerable amount of lycopene. The lycopene content of the other wild tomatoes was found to be lower than cultivated tomato. For

example, the green-fruited *S. habrochaites* [32] and *S. pennellii* [62] had lower lycopene content than *S. lycopersicum*. As a result, *S. pennellii* introgressions into cultivated tomato resulted in low lycopene content in introgression lines (ILs) [22,24,30,63]. A F_7 population derived from *S. lycopersicum* and *S. cheesmaniae* (orange-fruited wild tomato) also had lower lycopene but higher β-carotene content than commercial red-fruited hybrids Mountain Belle and Castlette [64].

Zeaxanthin and lutein are found at low levels in red-ripe tomato fruit [11]. Therefore, unlike our work, many studies did not measure these molecules. Although Kilambi et al. [17] used the same wild parental accession (LA 1589), we cannot directly compare results because of the use of different units. Interestingly, they did not detect zeaxanthin in *S. pimpinellifolium* while we quantified it as 3.6 mg g^{-1} DW in our study. Also, they could not detect zeaxanthin in *S. habrochaites,* but did in *S. cheesmaniae.* In the same study, the lutein content was higher in *S. cheesmaniae* than *S. habrochaites* followed by *S. pimpinellifolium.* In another study, lutein was quantified as 0.02 mg g^{-1} in Ailsa Craig [58] as compared to 3.25 mg g^{-1} in our cultivar, Tueza.

When our results and the literature are considered, not all wild species are obviously useful in breeding improved carotenoid content. However, *S. pimpinellifolium* clearly has great potential with its high lycopene content. In this work, although not all alleles that increased carotenoid content came from wild tomato, the major allele for increased lycopene (*lyc8.1*, PVE = 35 %) came from *S. pimpinellifolium*. Thus, introgression of this allele from *S. pimpinellifolium* into elite lines may provide enhanced lycopene content and redder tomato fruit.

4.1.2. Vitamins

Vitamins are another group of important antioxidant molecules. Of special importance are vitamin C and vitamin E. Vitamin C mainly acts as an enzyme cofactor while vitamin E scavenges lipid hydroperoxyl radicals [65]. We focused on the α isoform of vitamin E because this isoform is used to estimate the current Recommended Dietary Allowance (RDA) for vitamin E [66].

In other work, *S. pimpinellifolium* vitamin content was usually higher than that of cultivated tomato with values approximately two fold higher for vitamin C content [15,16,19]. In contrast, vitamin C content in our study was nearly the same in both parents and the population. Although vitamin C content displayed normal continuous variation in the population, some individuals had extreme alleles for vitamin C content suggesting that they are useful lines for improvement of this trait in cultivars.

When wild species were compared for their vitamin C content, *S. pimpinellifolium* was the best source followed by *S. peruvianum* and then *S. habrochaites* [13]. This previous work also found that the mean of the BC_2F_2 population derived from *S. habrochaites* and *S. lycopersicum* was lower than the value for the cultivated parent. These results agreed with the work done by Ökmen et al. [32], in which *S. habrochaites* had lower vitamin C content than *S. lycopersicum* and a derived backcross population. In a three species comparison, *S. pennellii* had the highest vitamin C content, followed by cultivated tomato (M82, Cervil, Levovil) and *S. habrochaites* with the only exception being *S. lycopersicum* cv. Ferum which had the lowest vitamin C level [25]. Supporting this finding, *S. pennellii* introgressions into M82 resulted in enhanced vitamin C content in two lines [12,22–24,67]. In contrast, Rousseaux et al. [30] could not detect vitamin C in *S. pennellii* and found that several introgression lines had lower vitamin C content than M82. On the other hand, they found that one line, IL12-4, had higher vitamin C content in agreement with previous work. Based on our results, *S. pimpinellifolium* was found to be a good source of vitamin C and many loci (66.6 %) for increased content were associated with wild alleles.

Unlike vitamin C, the literature has fewer studies on the genetic basis of vitamin E content in tomato. These studies used IL populations derived from *S. pennellii* introgressions in the M82 genetic background and found that IL 9-1 and IL 9-2-6 had higher vitamin E content than M82 [20,21]. In another study, vitamin E was quantified as 0.06 mg g^{-1} DW in commercial tomato Ailsa Craig at the full ripe stage [58]. In our study, the cultivated parent had 0.03 mg g^{-1} and *S. pimpinellifolium* had 5.6 fold higher vitamin E content than Tueza. The mean value of the population was close to the value for *S. pimpinellifolium* which indicates that the wild alleles were associated with high vitamin E content. Moreover, both major alleles for increased vitamin E *(vite9.3* and *vite10.1*) were associated with *S. pimpinellifolium* alleles. Thus, this wild species is a good source for the improvement of vitamin E content in cultivated tomato which could be bred by backcrossing and selection from high vitamin IBLs.

4.1.3. Glutathione

The reduced form of glutathione (GSH) has a pivotal role in antioxidant defense by removing many reactive species. Glutathione peroxidases catalyze the reduction of hydrogen peroxide by GSH into the oxidized form of glutathione (GSSG) and a water molecule [68]. Although glutathione is a powerful low molecular weight antioxidant molecule in the cell, it has been ignored in breeding studies. In the present study, *S. lycopersium* had higher levels of glutathiones than *S. pimpinellifolium*. GSH levels were higher than GSSG in both parents. GSSG is toxic to cells and must be reduced to GSH by glutathione reductase to maintain GSH/GSSG levels in the cell [68]. Di Matteo et al. [26] found a higher amount of both forms of glutathione in IL 7-3 derived from *S. pennellii* and M82. Unfortunately, we are unable to compare the results directly as they were expressed in different units (DW vs. FW). They also found higher GSSG levels than GSH in IL7-3. Thus, *S. pennellii* may not be a good source for improvement of glutathione levels in cultivated tomato. *S. pimpinellifolium* may have higher glutathione reductase activity than *S. pennellii*, thereby allowing it to maintain higher levels of GSH.

4.1.4. Phenolic acids

Phenolic acids are the largest and most diverse group of antioxidants. Because of their important biological activities in plants and health benefits to humans, phenolic acids have been the focus of plant breeding studies. In QTL studies, researchers mainly focused on total antioxidant capacity [e.g. 30,32] or total phenolic acid content [e.g. 32] because of the high positive correlation between antioxidant capacity and phenolic acid content [69].

In the present study, we quantified individual phenolic acids instead of total phenolic acid content. We examined individual compounds because phenolic acids are a huge, diverse class and different phenolic acids are synthesized by various plant species. Moreover, each phenolic acid may have distinct biological functions or health benefits [70–72]. The IBL population and its parents were quantified for 19 phenolic acids, including flavanols, flavones, flavonol-3-ols, hydroxybenzoic acids, hydroxycinnamic acids, and anthocyanins. The most abundant phenolic acid in *S. pimpinellifolium* was myricetin while gallic acid was the most abundant phenolic acid in *S. lycopersicum*. In addition, some genotypes of the IBL population had extreme quantities of syringic acid, chrysin, apigenin, catechin, vanillic acid, hydroxybenzoic acid, and malvidin as a result of transgressive segregation. In a few studies, chlorogenic acid was found to be the most abundant phenolic acid in *S. lycopersicum* cultivars and landraces [24,58,73,74]. In our study, chlorogenic acid was the most abundant phenolic acid after gallic acid in cultivated tomato. These findings confirm that individual phenolic content is highly dependent on both genotype and environmental factors.

Some studies showed that wild tomatoes had higher total phenolic acid content than cultivated tomatoes. For example, *S. pimpinellifolium* [16], *S. habrochaites* [32], and *S. pennellii* [30] had higher levels of phenolics acids than *S. lycopersicum*. Moreover, when multiple species were compared, *S. peruvianum* had highest phenolic content followed *by S. pimpinellifolium, S. habrochaites* and *S. lycopersicum* [13]. Our results agreed with these studies as we found higher phenolic acid content in *S. pimpinellifolium* than Tueza. Although alleles from both parents were

Table 4
QTLs that confirmed loci identified in previous studies.

Present Study Trait	QTL	Previous Studies Trait	Population, Species	Reference
Lycopene	*lyc9.1*	Lycopene	F2:3, *S. lyc*. x *S. pimp*.	[19]
	lyc8.1	Lycopene	Adv BC, *S. lyc*. x *S. neor*.	[75]
Carotene	*βcrn11.1*	β-Carotene	IBL, *S. lyc*. x *S.hab*.	[59]
	βcrn8.1	β-Carotene	Adv BC, *S. lyc*. x *S. neor*.	[75]
	βcrn11.1	β-Carotene	Adv BC, *S. lyc*. x *S. neor*.	[75]
Vitamin C	*Vitc10.1*	Vitamin C	Acc of *S. pimp* and *S.lyc*, RIL population from Cervil x Levovil	[76]
	vitc7.1	Vitamin C	RIL, Moneyberg x *S. penn*.	[15]
	vitc6.1	Vitamin C	BC2F6, *S. lyc*. x *S. hab*.	[32]
	vitc8.1	Vitamin C	RIL, Cervil x Levovil	[25]
	vitc10.2	Vitamin C	IL, M82 x *S. penn*	[25]
	vitc10.2	Vitamin C	BC, *S. lyc*. X *S. hab*.	[25]
Vitamin E	*vite5.2*	Enzyme in vitamin E biosynthesis (4-hydroxyphenyl pyruvate)	IL, M82 x *S. penn*.	[20]
	vite9.2	Vitamin E	IL, M82 x *S. penn*.	[21]
	vite9.2	Vitamin E	IL, M82 x *S. penn*.	[20]
	vite9.2	Enzyme in vitamin E biosynthesis (dimethyl-phytylquinone methyl transferase)	IL, M82 x *S. penn*.	[20]
	vite9.2	Enzyme in vitamin E biosynthesis (phytol kinase)	IL, M82 x *S. penn*.	[20]
	vite9.2	Enzyme in vitamin E biosynthesis (phospholipid transporter)	IL, M82 x *S. penn*.	[20]
Syringic acid	*sya8.1*	Total phenolics	IL, Moneyberg x *S. penn*.	[31]
	sya8.1	Total phenolics	IL, ILH - M82 x *S. penn*.	[27]
	sya8.1	Total phenolics	Landraces	[67]
	sya8.1	Total phenolics	IL, M82 x *S. penn*.	[30]
Chrysin	*cry8.1*	Total phenolics	IL ILH, M82 x *S. penn*.	[27]
	cry8.1	Total phenolics	IL, M82 x *S. penn*.	[30]
Apigenin	*apn9.1*	Total phenolics	IL, Moneyberg x *S. penn*.	[31]
Catechin	*ctn6.1*	Total phenolics	IL, M82 x *S. penn*.	[30]
	ctn7.1	Total phenolics	IL, M82 x *S. penn*.	[30]
Gallic acid	*ga8.1*	Total phenolics	IL ILH, M82 x *S. penn*.	[27]
	ga8.1	Total phenolics	IL, M82 x *S. penn*.	[30]
Vanillic acid	*va7.1*	Total phenolics	IL, M82 x *S. penn*.	[30]
Coumaric acid	*coa8.1*	Total phenolics	IL ILH, M82 x *S. penn*.	[27]
	coa8.1	Total phenolics	IL, M82 x *S. penn*.	[30]
Sinapic acid	*sa10.1*	Polyphenols	IL, M82 x *S. penn*.	[29]
	sa7.1	Total phenolics	IL, M82 x *S. penn*.	[30]

[a]BC: Backcross, IBL: Inbred backcross lines, IL: Introgression lines, ILH: Heterozygote introgression lines, RIL: Recombinant inbred lines, S. hab.: *Solanum habrochaites*, S. lyc.: *Solanum lycopersicum*, S.neor.: *Solanum neorickii*, S. penn.: *Solanum pennellii*, S. pimp.: *Solanum pimpinellifolium*.

associated with increased phenolic acid content, *S. pimpinellifolium* alleles may be helpful to increase both total phenolic acid content and individual phenolic compounds.

4.2. Confirmed QTLs and candidate genes

Because QTL identification is based on statistical analysis, there is always the chance of detecting false positive QTLs. However, comparison of the loci with previously identified QTLs in the literature and the annotated *S. lycopersicum* reference genome can be used to support a locus as a true positive and to discover potential candidate genes for each locus (Tables 3 and 4). Identification of candidate genes provides further support for the validity of the QTL and a better understanding of how the trait is controlled and how it can be more efficiently bred in tomato.

4.2.1. Carotenoids

Three QTLs were identified for lycopene content, two of which coincided with previously mapped loci. *Lyc8.1* (a major QTL with PVE = 35 %) was detected in a BC_2 population derived from *S. neorickii* [75]. *S. neorickii* alleles were associated with less lycopene content while *S. pimpinellifolium* alleles were associated with high lycopene content at this locus. The other QTL for lycopene, *lyc9.1*, matched one mapped in an $F_{2:3}$ population derived from *S. pimpinellifolium* (S0801) [19]. Interestingly, while *S. pimpinellifolium* alleles were associated with increased content in the previous work, *S. lycopersicum* alleles were responsible for increased content in our study. This discrepancy could arise from the use of different parental accessions and/or different population structures which yield unique allelic combinations.

Three loci were identified for β-carotene content, and two of these loci were also identified in previous studies. *βcrn8.1*, (a major QTL with PVE = 34 %) was detected by Fulton et al. [75]. Alleles from *S. pimpinellifolium* and *S. neorickii* had opposite effects on β-carotene content: wild tomato alleles were associated with high β-carotene only in our study. Another locus on chromosome 11, *βcrn11.1*, was identified in independent studies done by Fulton et al. [75], and Kabelk et al. [59] where the wild parents were *S. neorickii* and *S. habrochaites*, respectively. In both studies, wild alleles were associated with decreased β-carotene content, however, the *S. pimpinellifolium* allele increased content in our study. Because positive alleles for these major QTLs were coming from *S. pimpinellifolium*, this wild accession and individual IBLs can be useful to increase carotenoid content in new cultivars.

4.2.2. Vitamins

All QTLs detected for vitamin C, except the one on chromosome 12, were previously identified. *Vitc 6.1* was identified in *S. habrochaites* [32] and *vitc7.1* in *S. pimpinellifolium* (TO_937) [15]. Consistent with our study, wild alleles were associated with increased vitamin C content at these loci. *Vitc8.1* was detected in work done by Stevens et al. [25] in a RIL population obtained from crossing Cervil and Levovil. Both Cervil and *S. pimpinellifolium* are cherry tomatoes and alleles from these types were responsible for increasing vitamin C content at *vitc8.1*. In addition, a potential candidate gene for this vitamin C QTL was identified as glutathione peroxidase. Glutathione, vitamin C and vitamin E interact in a series of cyclical redox reactions [48]. Another locus, *vitc10.1*, corresponded to a QTL identified by Albert et al. [76] in different

tomato accessions including 10 *S. pimpinellifolium* lines. Similar to our results, the allele from cultivated tomato contributed to increased content. Stevens et al. [25] detected QTLs in *S. pennellii* and *S. habrochaites*-derived populations that overlapped with *vitc10.2* in our study. In all cases, wild alleles increased vitamin C content.

Only two of the five identified QTLs for vitamin E corresponded with loci detected in *S. pennellii* ILs, *vite5.2* [20] and *vite9.2* [20,21,77]. *Vite5.2* mapped to the region of 4-hydroxyphenylpyruvate dioxygenase [20]. This enzyme catalyzes the conversion of 4-hydroxyphenylpyruvate to homogentisate, a key step in vitamin E accumulation in plants. Moreover, vitamin E content and three components of vitamin E biosynthesis (dimethyl-phytylquinol methyl transferase, phytol kinase, and phospholipid transporter) mapped to a shared region of chromosome 9 (IL9-2) in the same study. In our study, *vite9.2* (a major QTL with PVE = 55 %) corresponded to this IL9-2 locus [20]. *S. pimpinellifolium* alleles were responsible for increased vitamin E content at this locus as in the case for the *S. pennellii* IL [20].

4.2.3. Glutathione

In our study, *S. lycopersicum* alleles were associated with increased content for all three GSH QTLs. Unfortunately, we cannot confirm our glutathione QTL results by comparison with previous studies because this trait has been neglected in other work. Further studies should be done to understand the genetic basis of glutathione and to identify associated loci.

4.2.4. Phenolic acids

Of the 43 QTLs identified for phenolic acids, 11 loci for eight phenolic compounds were previously identified and 10 loci were associated with candidate genes of which seven mapped to the reference genome. One of the four loci (*sya8.1*) for syringic acid corresponded to QTLs mapped in *S. pennellii* ILs [27,30], landraces [67], and *S. chimielewskii* ILs [31]. This previous work found that wild alleles decreased phenolic acid content [30,31] which is consistent with the association of *S. lycopersicum* alleles with increased content of the trait at this locus. Moreover, IL 8-2 and IL 8-2-1 were identified as hot spots for phenylpropanoid biosynthesis [27]. This pathway is involved in the biosynthesis of a large class of secondary metabolites, including flavonoids and phenolic acids, from aromatic amino acids [78]. In our work, three QTLs including ones for chrysin (*cry8.1*), gallic acid (*ga8.1*), and coumaric acid (*coa8.1*), were identified in the IL 8-2 region [27,30]. One of the four identified QTLs for the flavone apigenin on chromosome 9 (*apn9.1*) matched that from work done by Ballester et al. [31]. The *S. pennellii* allele at this locus was associated with low phenolic acid content in this previous work in agreement with the fact that the *S. pimpinellifolium* allele was associated with reduced apigenin content. In addition, this QTL localized to the same position as a mapped gene, flavanol synthase/flavanone 3-hydroxylase which plays a role in flavonoid biosynthesis. Two QTLs for catechin (*ctn6.1, ctn7.1*), one QTL for vanillic acid (*va7.1*), and one QTL for sinapic acid (*sa7.1*) corresponded to QTLs mapped by Rousseaux et al. [30], while one QTL for sinapic acid (*sa10.1*) matched a locus identified by Minutolo et al. [29] in *S. pennellii* ILs. In our work, alleles from *S. lycopersicum* were usually associated with increases in these phenolic acids in agreement with previous studies which showed that *S. pennelllii* alleles decreased the content. When we consider the results from our work and previous studies, wild alleles seem to be less promising than cultivated alleles for improvement of individual phenolic acids.

Possible candidate genes were identified close to some of the loci identified for phenolic acids. Dihydroflavanol-4-reductase genes at *ctn2.1*, *sa3.1*, and *chla12.1*; a cinnamoyl CoA reductase gene at *ectn1.1*, flavanone-3-hydroxylase at *ectn11.1*, genes for two forms of chalcone synthase at *va6.1* were identified based on their previously mapped positions in the tomato reference genome. These enzymes play roles in flavonoid biosynthesis. In addition to mapped genes, additional putative genes were described for some of the phenolic acid QTLs. These are enzymes in the phenylpropanoid pathway including a cinnamyl alcohol dehydrogenase gene and a hydroxycinnamoyl CoA quinate transferase gene at *cry8.1*, a cytochrome P450 NADPH reductase gene at *cna4.1*, and a dihydroflavanol-4-reductase gene at *sa3.1*. Other loci involved in the flavonoid pathway were a flavanol synthase/flavanone 3-hydroxylase gene at *ectn11.1* and a dihydroflavanol-4-reductase gene at *chla12.1*. Furthermore, three MYB-related transcription factors were identified around *chla12.1*. MYB genes are one of the largest classes of transcription factors and their proteins are reported to have a major role in phenylpropanoid biosynthesis [79].

5. Conclusion

Tomato contains high levels of antioxidant compounds. Thus daily consumption of tomato is beneficial to human health. In this study, we evaluated variation in antioxidant traits in an IBL population derived from cultivated tomato and *S. pimpinellifolium* acc. LA 1589. Transgressive segregation for the traits was observed and QTLs were identified for popular compounds including lycopene, β-carotene, vitamin C and E, as well as ignored molecules such as lutein, zeaxanthin, glutathione and individual phenolic acids. Our study showed that *S. pimpinellifolium* acc. LA 1589 alleles are good sources for improvement of antioxidant traits, including carotenoids, vitamins and some phenolic acids. Many of the loci colocalized with previously mapped QTLs and corresponded to locations in the tomato genome with genes involved in the relevant metabolic pathways. Thus, this work provides information that can be used for targeted breeding of antioxidant traits in tomato.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.plantsci.2019.110393.

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