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Exploring wild alleles from *Solanum pimpinellifolium* with the potential to improve tomato flavor compounds

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, İzmir 35433, Turkey
[b] MULTI Tohum, Antalya 07112, Turkey

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ABSTRACT

Most consumers complain about the flavor of current tomato cultivars and many pay a premium for alternatives such as heirloom varieties. Breeding for fruit flavor is difficult because it is a quantitatively inherited trait influenced by taste, aroma and environmental factors. A lack of genetic diversity in modern tomato cultivars also necessitates exploration of new sources for flavor alleles. Wild tomato *S. pimpinellifolium* and inbred backcross lines were assessed for individual sugars and organic acids which are two of the main components of tomato flavor. *S. pimpinellifolium* was found to harbor alleles that could be used to increase glucose and fructose content and adjust acidity by altering malic and citric acid levels. Single nucleotide polymorphism markers were used to detect 14 quantitative trait loci (QTLs) for sugars and 71 for organic acids. Confirmation was provided by comparing map locations with previously identified loci. Thus, seven (50 %) of the sugar QTLs and 22 (31 %) of the organic acids loci were supported by analyses in other tomato populations. Examination of the genomic sequence containing the QTLs allowed identification of potential candidate genes for several flavor components.

1. Introduction

Tomato (*Solanum lycopersicum*) fruit quality is explained by color, size and shape and sensory stimuli such as sweetness, acidity and flavor [1]. Flavor is a complex trait which is determined by both taste and olfaction (aroma compounds) and their interactions. In tomato, taste is mainly determined by sugars and organic acids whereas volatile compounds determine olfaction [2–4]. The types and quantities of sugars and their ratios to each other dictate sweetness which is the main determinant of quality and marketability of tomato fruits. Sugar content and variation in sugar types are highly genotype-dependent, and are also related to total soluble solids content, pH, titratable acidity and fruit size [5]. Organic acids affect fruit flavor by changing acidity. They regulate basic cellular processes such as modification of cellular pH and redox state [6]. Citric acid and malic acid are found in high concentrations in tomato and their contributions to tomato flavor are significant [7]. The other important component of flavor is volatile compounds, which contribute to aroma. Aroma depends on the composition and concentration of individual volatile compounds and the interactions between them [8].

The study of flavor is problematic because the metabolites contributing to flavor are greatly affected by environmental factors. In addition, high throughput assays for metabolite quantification can be difficult. In the past, these complications have dissuaded breeders from trying to improve tomato flavor [4]. Moreover, the pursuit of other breeding objectives (yield and resistance to stress) may have indirectly resulted in the loss of tomato flavor [9]. Breeding strategies started to consider flavor in the 1990s [10]. However, although QTLs have been identified for volatile and non-volatile compounds important to flavor, intensive breeding for these compounds has not yet been performed [3,11]. Lack of flavor in modern tomato cultivars is still a problem in the market [4].

In addition to poor flavor, many cultivars have limited genetic diversity when compared to the wealth of variation available in wild tomato germplasm [12]. It is estimated that cultivars contain only 5% of the genetic variation of their wild relatives [13]. Despite the use of sensitive molecular markers, very few polymorphisms have been identified in the cultivated tomato gene pool [14–18]. Reliance on single plant selection and limited germplasm during tomato domestication and breeding have decreased diversity in the crop. Tomato's self-pollinating nature has also contributed to this problem as genetic variation tends to decrease in inbreeding species even without artificial

Abbreviations: FID, flame ionization detector; IBL, inbred backcross line; RI, refractive index; RP, reverse phase; SNP, single nucleotide polymorphism

* Corresponding author.
E-mail addresses: nergizgurbuz@iyte.edu.tr (N.G. Çolak), neslihantek@iyte.edu.tr (N.T. Eken), mulger@multitohum.com (M. Ülger), annefrary@iyte.edu.tr (A. Frary), samidoganlar@iyte.edu.tr (S. Doğanlar).

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selection [10].

Wild relatives of tomato possess greater genetic diversity and have been examined to find new resources for *S. lycopersicum* improvement (e.g. [19,20]). For example, it is known that *S. pimpinellifolium* is useful for color and fruit quality traits and *S. chmielewskii* is useful for its high sugar content [21,22]. Breeders have tried to introduce some favorable traits from the wild relatives of tomato but this can take years of backcrossing and selection to remove deleterious wild alleles [10]. Traits successfully introgressed into cultivated tomato include biotic and abiotic stress tolerances [23–25] and fruit size and shape variation [26]. This approach pre-dates the use of transgenic methods [27] and metabolic engineering [28–32].

In the present work, alleles from the wild tomato species *S. pimpinellifolium* were explored for their potential to improve fresh market tomato flavor. *S. pimpinellifolium* was chosen for its known fruit quality traits and because it is the closest relative of cultivated tomato, *S. lycopersicum*. To this end, we performed targeted metabolic profiling of sugars and organic acids in an IBL population derived from a cross between the fresh market tomato cultivar, Tueza, and wild tomato species, *S. pimpinellifolium* LA 1589. Whole genome sequencing data from previous work [33] were used to identify quantitative trait loci (QTL) responsible for tomato flavor.

2. Materials and methods

2.1. Chemicals, instrumentation and plant material

Standard chemicals were analytical grade or min. 99 % purity (Applichem and Sigma) while those for high performance liquid chromatography (HPLC) were HPLC grade (VWR Chemicals). HPLC was done using a Shimadzu LC-20 AT model HPLC-RI with the HPLC column by GL Sciences (NH_2, 5 μm – 25 × 4.6 mm). Gas chromatography (GC) was performed using a Shimadzu GC 2010 Plus with a GC column by Restek (Rtx 5DA, 0.25 mm x 0.25 mm, 30 cm).

An interspecific IBL population derived from the cross *S. lycopersicum* cv. Tueza x *S. pimpinellifolium* (LA 1589) was used in the study. Tueza is a fresh market tomato cultivar with large (150–160 g), red, slightly flattened, round fruits. LA 1589 is a wild type tomato with small (1–10 g), red, round fruits. The IBL population and parents (ten plants per genotype) were grown in the field by Multi Tohum seed company (Antalya, Turkey). Ten plants per genotype were grown in double rows with 140 cm between wide rows and 50 cm between narrow rows. Within rows, plants were at 40 cm intervals. 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. All of the ripe fruits were harvested for each plant and 2 kg of ripe, unblemished, average-sized fruits were selected for further characterization as previously described [33].

2.2. Sample extraction

Samples (100 g) of tomato fruit were selected from the bulk harvest for the 94 individuals and two parents of the IBL population and were lyophilized. Fine powder was obtained from dried samples by grinding with a knife mill grinder. A total of 1 g of each freeze dried tomato sample was extracted in 5 ml hexane:dichloromethane (1:1, v:v) on an orbital shaker at 400 rpm, 18 °C overnight. Samples were centrifuged at 4 °C, 4000 rpm for 20 min. Supernatants were saved and pellets were subjected to extraction with the same solvent overnight once more. Samples were centrifuged at 4 °C and 4000 rpm for 20 min. Supernatants were combined, aliquoted and stored at − 80 °C until the analysis. Pellets were subjected to additional extraction with chloroform:methanol:water (1:3:1, v:v:v) on an orbital shaker at 400 rpm and 18 °C overnight. The procedure was the same as the hexane:dichloromethane extraction. Supernatants were combined, aliquoted and stored at − 80 °C.

2.3. Metabolite quantification

Each extract was analyzed for the quantification of the selected metabolites (sugars and organic acids) in duplicate. Glucose, fructose, and sucrose were analyzed with a modified isocritic method of HPLC-RI [34] using the chloroform:methanol:water (1:3:1, v:v:v) extract of tomato. Sugars were analyzed on an amino column (NH_2, 5 μm – 25 × 4.6 mm) at 40 °C using water: acetonitrile (10:90, v:v) as the mobile phase with a 1 ml min^{-1} flow rate. Sample injection was 20 μl with detection at 40 °C using positive mode with RI detector. Results were expressed as mg 100 g^{-1} DW.

The organic acids including citric acid, malic acid, tartaric acid, succinic acid, lactic acid, fumaric acid, butyric acid and shikimic acid were analyzed by derivitization with methoxamine hydrochloride and N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) using a combined, modified thermogradient GC-FID method [35,36]. The chloroform:methanol:water (1:3:1, v:v:v) extract of tomato was used for the other organic acids. Samples (100 μl) were vacuum evaporated at 30 °C. Each sample was dissolved in methoxamine hydrochloride (40 μl, 20 mg ml^{-1} in pyridine) in an ultrasonic bath for 5 min. Derivatization was at 37 °C for 90 min. Second derivatization was done with MSTFA. MSTFA (60 μl) was added to the sample which was incubated at 37 °C for 30 min. The derivatized sample was centrifuged at 14.000 rpm, 5 min. The supernatant was injected into GC-FID.

Organic acids were then analyzed on an Rtx 5DA (0.25 mm × 0.25 mm, 30 m) column with a thermogradient program. The column temperature was programmed from 100 °C (1 min held) to 150 °C at a rate of 5 °C min^{-1}, from 150 °C (1 min held) to 280 °C at a rate of 5 °C min^{-1}, 2 min hold at the final temperature was applied. Injection port temperature was held at 250 °C, detector temperature was held at 300 °C. Carrier gas was nitrogen (N_2) and split ratio was 1/25. Detection was done by FID. Results were expressed as mg 100 g^{-1} DW.

2.4. Genomic data and QTL mapping

Genomic data were obtained from previous work that performed genotyping by sequencing (GBS) of the population [33]. A total of 3125 genome-wide SNP loci were used in QTL mapping of the fruit quality traits. Log 2 transformation was performed for metabolic data. QGene version 4.0 [37] was used for QTL analysis. The composite interval mapping (CIM) QTL analysis method was performed with automatic forward cofactor selection, a scan interval of 0.2 Mb and a LOD threshold ≥ 3 for QTL identification. To determine if the identified QTLs coincided with previously mapped loci, the loci's nearest marker map positions were compared and an overlap was assumed if the candidate gene fell within the interval encompassed by markers significantly linked to the trait. Candidate genes around the identified QTLs associated with each trait were identified from the Sol Genomics website. The full gene list in the QTL region plus the 0.1 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 sugars and organic acids

A total of 94 IBL individuals and parental accessions (cultivated parent Tueza and wild parent LA 1589) were characterized for three sugars including glucose, fructose and sucrose; and eight organic acids including citric acid, malic acid, tartaric acid, lactic acid, fumaric acid, butyric acid, salicylic acid and shikimic acid.

Table 1
Sugar and organic acid content measured in IBL population and parents: Tueza and LA 1589. Quanitities of metabolites are given as mg 100 g^{-1} DW.

Metabolite	Parents		IBL Population		
Sugars	Tueza	LA 1589	Mean	Range	CV%
Glucose	8738.04	4153.29	6596.45	0–9897.50	24.0
Fructose	8401.38	3967.70	5839.52	0–9457.71	28.0
Organic acids					
Salicyclic acid	0.01	0.02	0.03	0 − 0.50	286.8
Lactic acid	0.31	0.04	0.18	0 − 1.35	98.0
Malic acid	6.19	0.86	3.58	0 − 27.0	100.7
Shikimic acid	0.94	0.00	1.17	0 − 3.78	89.8
Citric acid	10.40	8.51	7.59	0 − 20.60	61.9

3.1.1. Sugars

Sucrose, glucose and fructose were analyzed in the tomato fruits. Sucrose was not detected in fruits of either parent or in the population. This result indicates that either sucrose was not present or that its level was below the limit of detection (100 ppm) of the HPLC technique used for quantification. The cultivated parent Tueza had two-fold higher glucose and fructose content than LA 1589. The mean values for both traits in the population were intermediate between the two parents. Both glucose and fructose displayed continuous and nearly normal variation in the population (Fig S1, Table 1). Some individuals appeared to have no glucose or fructose which could, again, indicate that the level of sugar was below the limit of detection.

3.1.2. Organic acids

Citric, malic, tartaric, lactic, fumaric, butyric, salicylic and shikimic acids were analyzed in the tomato fruits. Tartaric acid, fumaric acid and butyric acid were not detected in the parents or in the IBLs. Citric acid was the most prevalent organic acid in the fruits. The parents had high citric acid content with 2 mg 100 g^{-1} DW more citric acid in Tueza than in LA 1589: 10.4 vs. 8.5 mg 100 g^{-1} DW (Table 1). Interestingly, while the population had a mean citric acid content that was lower than both parents, some of the IBLs had very high values ranging up to 20.6 mg 100 g^{-1} DW. Tueza had seven-fold higher malic acid content than LA 1589 which had a very low level of this acid, 0.86 mg 100 g^{-1} DW. The mean amount of malic acid in the population was intermediate. The parents did not have high amounts of other organic acids. Organic acids displayed continuous variation in the population (Fig S2).

3.2. QTL mapping and candidate genes

3.2.1. Sugars

A total of 14 QTLs were identified for sugars based on a LOD threshold of 3 (Table S1). Six QTLs were identified for glucose including five QTLs on chromosome 5 and one QTL on chromosome 9 with the percentage of phenotypic variation explained (PVE) varying between 16 and 29 %. Four of the QTLs on chromosome 5 had minor effects with PVE < 20 %. However, *glc5.1* had a moderate effect with a PVE of 23 %. The QTL (*glc9.1*) on chromosome 9 also had moderate effect with a PVE of 29 %. At every locus, the LA 1589 alleles contributed to increased glucose content. Eight QTLs were identified for fructose including five on chromosome T5 and one each on chromosomes 3, 9 and 11, PVEs varied between 15 and 29 %. All of the loci had minor effects except for *frc5.1*and *frc9.1* which had moderate effects (PVE values of 23 and 29 %). For all but two of the loci (*frc3.1*, *frc11.1*), the LA 1589 alleles contributed to increased fructose content.

3.2.2. Organic acids

A total of 71 QTLs were identified for organic acids based on a LOD threshold of 3 (Table S2). Thirteen QTLs were identified for salicylic acid with PVEs varying between 22 and 48 %. Seven of the loci were considered to be major QTLs as they had PVEs >30 %; *sca3.1,* had the highest magnitude of effect, 48 %. The remaining QTLs had moderate effects. Seven of the QTLs had alleles for increased salicylic acid from Tueza while six had LA 1589 alleles for increased content.

Fourteen QTLs for lactic acid were identified with the PVEs varying between 20 and 48 %. Major QTLs were identified on chromosomes 1, 3, 7, and 11 with *la1.3* having the greatest effect. *La7.2* was also noteworthy as it had a 40 % magnitude of effect. The other identified QTLs showed moderate effects. Half of the QTLs had alleles for increased lactic acid content from Tueza while the other half had alleles from LA 1589.

Eleven QTLs were identified for malic acid on six different chromosomes, with multiple loci on chromosomes 1, 3, and 7 and PVEs ranging from 19 to 57 %. Three of the identified QTLs were major QTLs with *ma1.2* having the greatest magnitude of effect. Five QTLs had moderate effect and only one was a minor QTL. LA 1589 alleles were associated with increased malic acid for six loci while Tueza alleles were associated with higher acid content for the remaining five loci.

Sixteen QTLs for shikimic acid were detected with the PVEs varying between 22 and 46 %. Six of the identified QTLs were major QTLs with *sha1.2*, *sha8.1*, and *sha9.1* having PVEs greater than 40 %. Nine QTLs had moderate effects and one had minor effects. Increased shikimic acid content for the majority of the QTLs (75 %) was associated with allelic effects from Tueza.

A total of 17 QTLs for citric acid were detected with the PVEs varying between 19 and 59 % (Table S2). Ten of the QTLs had major effects with PVEs > 30 %. QTLs *cca9.2*, *cca6.4* and *cca3.2* had the greatest magnitudes of effect ranging from 41 to 59 %. Five and two QTLs had moderate and minor effects, respectively. Nine of the loci had alleles from Tueza which increased citric acid content while the remaining eight loci had LA 1589 alleles associated with increased acidity.

3.3. Colocalized traits

Many of the QTLs for the sugar and organic acid traits were colocalized on tomato chromosomes (Table S3). For example, loci controlling glucose and fructose content were colocalized multiple times on chromosome 5 and once on chromosome 9. Organic acid QTLs mapped together 19 times on different chromosomes. Because there are so many colocalizations, only cases in which at least three QTLs mapped together will be presented here by chromosome number.

Lactic, citric and malic acid QTLs colocalized on chromosome 1 at 26.6 Mb position. In addition, QTLs for all five organic acids were colocalized at 76.6 Mb on chromosome 1. There were QTLs for lactic, malic and shikimic acids as well as a locus for fructose on chromosome 3 (38 Mb). Chromosome 3 also contained loci for lactic, malic and citric acids at 56 Mb. On chromosome 5, QTLs for glucose, fructose, and shikimic acid colocalized at both 40.1 and 42.1 Mb. At 38.8 Mb position on chromosome 6, there were QTLs for lactic, malic and citric acid. On chromosome 7, QTLs for four organic acids (except shikimic acid) mapped to the same position at 0.2 Mb. On chromosome 11, loci for three organic acids mapped together at 0.3 Mb. Another cluster was found further down the chromosome (2.3 Mb) and contained QTLs for fructose, shikimic acid, and lactic acid. Such colocalizations are expected given the intersecting nature of metabolic pathways.

4. Discussion

Tomato breeding objectives have shifted somewhat from high yielding, resistant cultivars to nutrient and flavor-rich cultivars as a result of consumer preferences [4]. Despite advances in molecular techniques and markers, breeding studies for fruit flavor are difficult because these traits are polygenic and strongly affected by environmental factors [38]. In addition, flavor is a complex, somewhat subjective trait influenced by taste and aroma. Taste is determined by sweetness and sourness which are influenced by soluble sugars and

acids, respectively [39–42], and aroma is determined by volatile compounds [4,43]. Besides being essential to fruit flavor, sugars and organic acids constitute over 60 % of dry matter and contribute to soluble solids content (SSC) in tomato [44,45]. In addition to their contributions to flavor, soluble sugars, acids and potassium salts determine osmotic pressure, which induces turgor pressure resulting in fruit cell expansion. Thus, sugar and acid contents are important in regulation of fruit size by controlling osmotic pressure [46]. High sugar content is a favorable trait in tomato breeding because of its contributions to fruit sweetness [47–50]. Organic acid content is important in determining the balance between sweetness and acidity.

4.1. Wild germplasm as a source for flavor improvement

4.1.1. Sugars

To date, many QTL and breeding studies have evaluated sugar content by total SSC instead of determining individual sugar levels. Thus, QTL mapping studies focusing on individual sugar content are more limited in the tomato literature. In the present work, an IBL population developed from backcrosses between LA 1589 and Tueza was evaluated for three simple sugars: glucose, fructose and sucrose in order to identify associated QTLs. Parental alleles for glucose and fructose content were extreme and Tueza contained about two-fold more glucose and fructose than LA 1589. Glucose and fructose were at similar levels in each line. Sucrose was not detected in the parents or in the population. This result was expected because *S. lycopersicum* and *S. pimpinellifolium* have high activities of acid invertases [51–53] at the late stages of fruit development [48,49,54]. Invertases hydrolyze sucrose into glucose and fructose. Therefore these species accumulate hexose sugars in their ripe fruit instead of sucrose [55]. Interestingly, *S. peruvianum*, *S. habrochaites S. chmielewskii* were reported to be sucrose accumulators [56,57].

In contrast to the current results, Schauer et al. (2004) [58] indicated that *S. pimpinellifolium* contained high levels of sugars and sugar alcohols such as fructose, glucose, raffinose, galactose, glycerol, rhamnose, isomaltose and mannose, but low levels of fucose, inositol, maltose and xylose as compared to cultivated tomato (M82). This discrepancy is most likely due to the difference in *S. lycopersicum* cultivars used in the studies. M82 is a field-grown processing tomato whereas Tueza is a greenhouse fresh market cultivar which is probably sweeter than M82. Unfortunately, it is not possible to compare our results directly because they are expressed in different units (dry vs fresh weight). *S. pennellii* LA716 was also shown to contain much more glucose and fructose than *S. lycopersicum* M82 while *S. chmielewskii*, *S. habrochaites* and *S. neorickii* had much lower levels [58]. However, it must be remembered that unfavorable phenotypes for complex traits can often mask the contributions of individual beneficial alleles [12]. Indeed, in the current work, although LA 1589 fruit had lower sugar content than Tueza fruit, the majority of alleles (86 %) for increased glucose and fructose content QTLs were from the wild species LA 1589. This result also highlights the advantage of using advanced backcross populations such as IBLs for QTL detection when the ultimate goal is introgression of favorable traits from wild germplasm [59]. The feasibility of using wild tomato for fruit quality enhancement is exemplified by the successful commercial tomato hybrids that carry introgressions from *S. pennellii* for increased fruit SSC [60].

4.1.2. Organic acids

In previous work, organic acid content was most often evaluated by titratable acidity. Those studies that measured individual acids were confined to measuring a few main ones such as citric, malic and ascorbic acids [58,61–64]. The current work tested for eight organic acids; five were detected. Citric and malic acids were most prevalent in both cultivated tomato and *S. pimpinellifolium* and minor or indetectable amounts of shikimic, lactic and salicylic acids were found. Both citric and malic acids are major contributors to tomato flavor. In addition to its contribution to acidity, malic acid is involved in starch metabolism [65]. A high concentration of malic acid in the fruit is associated with reduced soluble sugars content. Overall, wild fruits were less acidic than Tueza ones. Compared to Tueza which had 1.7-fold less malic than citric acid, the wild parent had nearly 10-fold less malic than citric acid. In contrast to *S. pimpinellifolium*, *S. neorickii*, *S. chmielewskii* and *S. pennellii* had much more malic acid than cultivated tomato M82. Moreover these other wild species had higher relative concentrations of malate to citrate [58]. This difference is probably due to the fact that *S. lycopersicum* and *S. pimpinellifolium* are the only red-fruited species and it is known that malic and citric acids are rapidly interconverted in green fruit but that little citric acid is converted to malic acid in red fruit [66]. The similar acidity profile of these two species suggests that *S. pimpinellifolium* and its lower malic acid content may be more useful than other wild germplasm for improvement of cultivated tomato flavor by alterations in acidity and soluble solids content. Based on our results, *S. pimpinellifolium* was rich in alleles that could be used to increase or decrease individual organic acids with a major malic acid QTL (PVE = 57 %) on chromosome 1 and citric acid QTLs (PVE = 41–59%) on chromosomes 3, 6 and 9.

4.2. Confirmed QTLs and candidate genes

The identification of QTLs is based on statistical analyses and each locus has an associated probability of being a false positive. Therefore, it is important to use multiple sources of data or information to confirm a given locus before it is considered for use in breeding. For this reason, the map positions of all QTLs identified in this study were compared with previously described QTLs and with the annotated *S. lycopersicum* genome sequence to discover potential candidate genes responsible for the loci (Tables 2 and 3). Some of these candidates corresponded to previously mapped genes while others matched genes with putative functions in related pathways.

4.2.1. Sugars

A total of six QTLs were identified for glucose content, two of which coincided with previously mapped loci. *Glc5.1* was detected in *S. pennellii* ILs [30] and in a *S. neorickii* BC_2 population [62] while *glc9.1* was also identified by Fulton et al. (2000) [62]. A candidate gene was identified for *glc9.1*, UDP-D-glucuronate 4-epimerase. This enzyme is involved in the synthesis of hemicelluloses, important components of the cell wall, which are synthesized from glucose. Three of the chromosome 5 glucose QTLs also had potential candidate genes. *Glc5.4* seems to have the most direct effect on glucose content because it corresponded to a phosphoglucomutase associated with glucose degradation. Because the *S. pimpinellifolium* allele for *glc5.4* was associated with higher glucose levels, we hypothesize that this allele is associated with lower phosphoglucomutase activity. In addition, *glc5.2* and *glc5.5* mapped to locations with genes that have potential roles in carbohydrate metabolism. *Glc5.2* was proximate to three potential candidate genes: a glucoronyl transferase, a glucan hydrolase and a cellulose synthase. *Glc5.5* may correspond to one or two functions that mapped in the region: glucosyl transferase and/or glucoside hydrolase. In every case, the wild alleles for the QTLs/candidate genes contributed to increased glucose content. Thus, the potentially positive effects of these alleles on tomato sweetness could be useful in breeding more flavorful tomatoes.

Eight loci were identified for fructose content; five of which were also detected in previous studies. For example, *frc5.1* was identified in *S. neorickii* [62] as well as *S. pennellii* [30]. *Frc5.2* and *frc5.5* were both detected in *S. pennellii* ILs [30,67] and *frc9.1* was also present in *S. neorickii* [62]. All of these chromosome 5 and 9 loci had wild alleles which were associated with increased fructose content. The wild allele for *frc11.1* was associated with reduced fructose, a relationship which was also observed in *S. pennellii* by Schauer et al. (2006) [30]. None of the fructose QTL were located in regions that overlapped with known

Table 2
Flavor QTLs that confirmed loci identified in previous studies.

Present study		Previous studies		
Trait	QTL	Trait	Population - Species[a]	Reference
Glucose	*glc5.1*	Soluble solids content	BC2 - *S. lyco./S. neor.*	[62]
	glc5.1	Glucose	IL - *S. lyco./S. penn.*	[30]
	glc9.1	Soluble solids content	BC2 - *S. lyco./S. neor.*	[62]
Fructose	*frc5.1*	Soluble solids content	BC2 - *S. lyco./S. neor.*	[62]
	frc5.1	Fructose	IL - *S. lyco./S. penn.*	[30]
	frc5.2	Fructose	IL - *S. lyco./S. penn.*	[30]
	frc5.5	Fructose	IL, ILH - *S. lyco./S. penn.*	[67]
	frc5.5	Fructose	IL - *S. lyco./S. penn.*	[30]
	frc9.1	Soluble solids content	BC2 - *S. lyco./S. neor.*	[62]
	frc11.1	Fructose	IL - *S. lyco./S. penn.*	[30]
Salicylic acid	*sca1.1*	Salicylic acid	IL - *S. lyco./S. penn.*	[68]
	sca2.3	pH	BC2 - *S. lyco./S. neor.*	[62]
	sca9.1	Total organic acids	BC2 - *S. lyco./S. neor.*	[62]
	sca9.1	Total acids	BC2 - *S. lyco./S. neor.*	[62]
	sca9.1	pH	BC2 - *S. lyco./S. neor.*	[62]
	sca9.1	Salicylic acid	IL - *S. lyco./S. penn.*	[68]
	sca10.1	Salicylic acid	IL - *S. lyco./S. penn.*	[68]
	sca10.2	Salicylic acid	IL - *S. lyco./S. penn.*	[68]
Malic acid	*ma1.1*	Malic acid	Heirloom varieties	[4]
	ma1.2	Malic acid	IL - *S. lyco./S. penn.*	[68]
	ma1.2	Malic acid	BC4 - *S. lyco./S. peru.*	[70]
	ma1.3	Malic acid	IL - *S. lyco./S. penn.*	[30]
	ma1.3	Malic acid	BC3 - *S. lyco./S. habr.*	[70]
	ma1.3	Malic acid	BC3 - *S. lyco./S. neor.*	[70]
	ma3.2	Malic acid	Heirloom varieties	[4]
	ma3.3	Malic acid	Heirloom varieties	[4]
	ma6.1	pH	BC2 - *S. lyco./S. neor.*	[62]
	ma7.1	Malic acid	IL - *S. lyco./S. penn.*	[30]
	ma9.1	pH	BC2 - *S. lyco./S. neor.*	[62]
Lactic acid	*la6.1*	pH	BC2 - S. lyco. / S.neor.	[62]
Shikimic acid	*sha3.2*	Total acids	BC2 - *S. lyco./S. neor.*	[62]
	sha5.1	Shikimic acid	IL - *S. lyco./S. S. penn.*	[30]
Citric acid	*cca2.1*	Citric acid	IL - *S. lyco./S. penn.*	[30]
	cca3.3	Citric acid	Heirloom varieties	[4]
	cca6.3	Citric acid	RIL - *S. lyco./S. pimp.*	[71]
	cca6.4	pH	BC2 - *S. lyco./S. neor.*	[62]
	cca7.1	Citric acid	IL - *S. lyco./S. penn.*	[72]
	cca8.1	Citric acid	IL - *S. lyco./S. penn.*	[72]

[a] BC: Backcross, IL: Introgression lines, ILH: Heterozygote introgression lines, RIL: Recombinant inbred lines, S. habr.: *Solanum habrochaites*, S. lyco.: *Solanum lycopersicum*, S.neor.: *Solanum neorickii*, S. penn.: *Solanum pennellii*, S.peru.: *Solanum peruvianum*, S. pimp.: *Solanum pimpinellifolium*.

genes for carbohydrate metabolism.

4.2.2. Organic acids

QTLs were detected for five organic acids: salicylic, lactic, malic, citric and shikimic acids. Four of the 13 salicylic acid loci, *sca1.1*, *sca10.1*, *sca10.2*, were previously identified in the *S. pennellii* ILs [68]. Interestingly, the *S. pennellii* alleles for these loci were associated with less salicylic acid whereas the *S. pimpinellifolium* alleles caused increased amounts of this acid. The region containing *sca1.1* has one possible candidate gene: dehydroquinate dehydratase/shikimate:NADP oxidoreductase, an enzyme in the shikimic acid pathway which synthesizes chorismate, a precursor of salicylic acid. *Sca2.3* and *9.1* also overlapped with total organic acids, total acids and pH QTLs in a *S. neorickii* BC2 population [62]. Moreover, *sca 11.2* overlapped with a QTL for titratable acidity in a RIL population derived from tomato breeding lines, K03 and K09 [69]. Only one of the 14 lactic acid loci corresponded to a previously mapped pH QTL. *La6.1* coincided with a chromosome 6 locus in the *S. neorickii* population [62]. The *S. pimpinellifolium* and *S. neorickii* alleles had opposite effects on lactic acid content.

Malic and citric acid are significant contributors to tomato acidity and hence, flavor [7]. Thus, they are useful targets for alteration of these traits. In addition, malic acid has a regulatory role in starch metabolism which affects soluble solids content [65]. Nine of the 11 malic acid QTLs were previously identified by other researchers. Fulton et al. (2000) [62] detected two loci affecting pH that overlapped with *ma6.1* and *ma9.1*. However, the allelic effects for *S. neorickii* and *S. pimpinellifolium* differed, *S. neorickii* was associated with increased acidity while *S. pimpinellifolium* alleles were responsible for decreased malic acid content. Three of the QTLs corresponded with loci detected in *S. pennellii* ILs, *ma1.2*, *ma1.3*, *ma7.1* [30,68] and in *S. peruvianum* (*ma1.3*, [70]. In only one case, the wild alleles had similar effects on malic acid content. An additional three loci overlapped with those found by Tieman et al. (2017) [4] in heirloom tomatoes, and one corresponded to loci found in both *S. habrochaites* and *S. neorickii* [70]. Six of the 17 citric acid QTLs were previously identified. Capel et al. (2015) [71] identified a locus at the same position as *cca6.3* in *S. pimpinellifolium* recombinant inbred lines. In both studies the wild allele was associated with increased citric acid content. This QTL also overlapped with a titratable acidity QTL in Kimbara's work [69]. Other loci corresponded to those identified by Tieman et al. (2017) [4], Schauer et al. (2006) [30], Fulton et al. (2000) [62], and Causse et al. (2004) [72]. No consistent relationships were observed for wild versus cultivated parent alleles. Four of the newly identified citric acid QTLs had potential candidate genes. *Cca3.1* was located near a mapped chloroplast malate dehydrogenase gene. This enzyme is involved in the TCA cycle and catalyzes the conversion of malate to oxaloacetate which is then converted to citric acid. The other three QTLs, *cca1.2*, *cca3.2* and *cca9.2*, localized near genes involved in amino acid metabolism using TCA cycle intermediates.

Shikimic acid is an important molecule which plays a role in the synthesis of flavonoids [73,74], isoquinoline alkaloids, anthocyanins, terpenoids [74] and aromatic amino acids [75]. Only two of the 16 shikimic acid QTLs overlapped with previously identified loci, *sha3.2* [62] and *sha5.1* [30]. For *sha3.2*, cultivated tomato alleles were associated with increased acidity whereas, for *sha5.1*, *S. pimpinellifolium* and *S. pennellii* alleles had opposite effects. Two shikimic acid loci mapped to the genome near potential candidate genes. *Sha5.3* mapped to a region containing a cluster of caffeoyl-CoA O-methyltransferase genes which have roles in chlorogenic acid and phenylpropanoid biosynthesis. Similarly, *sha10.1* co-localized with a different O-methyltransferase on chromosome 10. A citric acid QTL on chromosome 3, *cca3.1*, mapped to the same region as chloroplast malate dehydrogenase, an enzyme in the TCA cycle. Three additional citric acid loci corresponded to genes involved in the synthesis of amino acids from TCA cycle intermediates.

5. Conclusion

The genetic control of tomato quality has been studied for many years, however, these studies were mostly limited to morphological and disease resistance traits. As a result, the genetic and molecular bases of natural variation of tomato metabolites that affect flavor are still far from being clearly understood. In the present study, we have identified loci for sugar and organic acid content and suggest that *S. pimpinellifolium* alleles can be used to improve flavor traits such as sweetness and acidity in cultivated tomato thus confirming the high breeding potential of *S. pimpinellifolium*.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

Table 3
Possible candidate genes for the flavor QTLs identified in the study.

Trait	QTL	Solgenomics ID	Position	Mapman (General class)
Glucose	*glc5.2*	Solyc05g016320.1	15.55	UDP glucosyl and glucoronyl transferases
	glc5.2	Solyc05g016390.2	16.03	Beta 1,3 glucan hydrolases
	glc5.2	Solyc05g016470.1	17.08	Cell wall-cellulose synthesis
	glc5.4	Solyc05g026490.2	41.50	Glycolysis-plastid branch, phosphoglucomutase
	glc5.5	Solyc05g047560.1	59.03	UDP glucosyl and glucoronyl transferases
	glc5.5	Solyc05g049980.2	59.82	Cell wall-degradation, pectate lyases and polygalacturonases
	glc9.1	Solyc09g082990.2	68.69	Cell wall-precursor synthesis
Salicyclic acid	*sca1.1*	Solyc01g067750.2	76.69	Amino acid metabolism-synthesis of aromatic amino acids, chorismate - dehydroquinate/shikimate dehydrogenase
Shikimic acid	*sha5.3*	Solyc05g041270.1	50.95	Specialized metabolism-phenylpropanoids, lignin biosynthesis
	sha5.3	Solyc05g041300.1	51.09	Specialized metabolism-phenylpropanoids, lignin biosynthesis
	sha5.3	Solyc05g041310.1	51.20	Specialized metabolism-phenylpropanoids, lignin biosynthesis
	sha5.3	Solyc05g026330.1	40.89	Specialized metabolism-phenylpropanoids, lignin biosynthesis
	sha5.3	Solyc05g026350.1	40.92	Specialized metabolism-phenylpropanoids, lignin biosynthesis
	sha5.3	Solyc05g041320.1	51.20	Specialized metabolism-phenylpropanoids, lignin biosynthesis
	sha5.3	Solyc05g041270.1	50.95	Specialized metabolism-phenylpropanoids, lignin biosynthesis
	sha5.3	Solyc05g041300.1	51.09	Specialized metabolism-phenylpropanoids, lignin biosynthesis
	sha5.3	Solyc05g041310.1	51.20	Not assigned-unknown
	sha5.3	Solyc05g026330.1	40.89	Specialized metabolism-phenylpropanoids, lignin biosynthesis
	sha5.3	Solyc05g026350.1	40.92	Specialized metabolism-phenylpropanoids, lignin biosynthesis
	sha5.3	Solyc05g041320.1	51.2	Specialized metabolism-phenylpropanoids, lignin biosynthesis
	sha10.1	Solyc10g005060.2	53.3 − 56.27	Specialized metabolism-phenylpropanoids, lignin biosynthesis
Citric acid	*cca1.2*	Solyc01g066480.2	74.48	Amino acid metabolism-degradation of aromatic amino acids, tyrosine
	cca3.1	Solyc03g071590.2	18.25	TCA / organic acd transformation - other organic acid transformaitons, cyt MDH
	cca3.2	Solyc03g077920.1	48.53	Amino acid metabolism-synthesis of aromatic amino acids, tryptophan
	cca9.2	Solyc09g091470.2	70.74	Amino acid metabolism-degradation of branched-chain group.

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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.2020.110567.

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