Evaluation of a Subset of the *Solanum lycopersicum* var. *cerasiforme* Core Collection for Horticultural Quality and Fruit Phenolic Content

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Abstract. One hundred *S. l.* var. *cerasiforme* (Dunal) accessions from the ‘Tanksley’ designated core collection were evaluated for horticultural quality under greenhouse conditions. Fourteen selected accessions were grown under field conditions in a replicated trial to evaluate the fruit for phenolic content. Total fruit phenolics ranged from 44 to 82 mg/100 g gallic acid equivalents (GAE) fresh weight (FW) as measured by Folin-Ciocalteu assay (F-C), and 12 to 108 mg/100 g FW as measured by high-performance liquid chromatography (HPLC). Five accessions (LA1712, LA1455, LA2633, LA1668, and LA2632) had significantly higher total phenolics (F-C) than cultivars ($P \leq 0.05$). These five accessions also possessed interesting phenolics profiles, including high levels of caffeoylquinic acids (LA1620, LA1455, and LA2633) and rutin (LA2633).

Tomatoes are an important food crop and a significant constituent in the human diet, being the second most consumed vegetable in the United States after potatoes (Economic Research Service, USDA, 2008). Because they comprise a large portion of the average vegetable intake, tomatoes are an important source of phenolics in the human diet. Phenolics are a group of secondary plant metabolites that include phenolic acids, anthocyanins, and flavonoids. These compounds are produced only by plants and exhibit a broad range of health-promoting effects when consumed in foods of plant origin. Phenolics have been correlated with anti-inflammatory, antiviral, and antiallergenic effects (Kushad et al., 2003; Middleton and Kandaswami, 1993). Phenolics are also strong antioxidants, but recent research

indicates that phenolics do not act as antioxidants in vivo (Lotito and Frei, 2006) suggesting their health benefits are derived from an unidentified mechanism.

Compared with many vegetables, tomato phenolic levels are relatively low, ranking 15th in total phenolics of 23 vegetables (Vinson et al., 1998). The highest concentrations of these compounds are found in the skin of the fruit with lesser amounts found in the flesh (Kushad et al., 2003; Stewart et al., 2000). Considerable effort has been made to increase the levels of phenolics in tomato fruit through the use of genetic engineering (Schijlen et al., 2004, 2006) and introgression of genes from wild species (Willits et al., 2005). However, the use of genetic engineering is limited by public concerns over the safety of genetically modified foods, whereas the introgression of novel traits from wild species is often hindered by linkage of undesirable traits.

The feral or wild tomato subspecies *Solanum lycopersicum* var. *cerasiforme* ranges throughout much of South and Central America and represents the closest wild gene pool for the introgression of novel traits into cultivated tomato. Many *S. l.* var. *cerasiforme* accessions are similar in morphology to cultivated tomato; however, this subspecies has previously been reported to contain high levels of total phenolics (Hanson et al., 2004). Steve Tanksley designated a set of 100 *S. l.* var. *cerasiforme* accessions as a core collection, which was assembled to maximize sampling of geographical and morphological diversity (S. Tanksley, Cornell University, personal communication). These accessions will be extensively genotyped using molecular markers and the information made publicly available (some marker information was already available by request). Using this core set of genotypes that

has been previously characterized at the molecular level will facilitate introgression of these genes related to phenolic content into cultivars. The objective of this research was to evaluate tomato accessions for phenolic content with the aim of selecting accessions that could be crossed to elite lines for the development of high-phenolic tomato cultivars.

Materials and Methods

Plant material. One hundred accessions of *S. l.* var. *cerasiforme* were obtained from Steve Tanksley of Cornell University (personal communication). For the full list of accessions in the Tanksley core, see Boches (2009). Because some of these accessions originate from cross-pollinated, heterozygous populations, Tanksley proposed selfing them to homozygosity to provide a more permanent genetic resource amenable to genetic analysis. Seed was obtained from Tanksley in the S1 generation. All 100 accessions were evaluated under greenhouse conditions for horticultural qualities such as earliness and yield in the S1 generation. Greenhouse growth of tomato plants was as for Mes et al. (2008). In brief, tomato seeds were sown in Sunshine SB40 professional growing mix (Sun Gro Horticulture, Bellevue, WA) in 5-cm-diameter plastic transplant cells. Seedlings were transplanted into 3.8-L pots filled with Special Blend potting mix and 20 g of 14N–6.1P–11.6K slow-release fertilizer (Simplot 14-14-14) after 3 weeks. Plants were given 10 g of supplemental fertilizer after 4 weeks in the pots. Greenhouse temperature was set to 18 °C night and 25 °C day. Supplemental lighting was provided for 16 h per day by 400-W metal halide and 400-W high-pressure sodium Sun System 3 high-intensity discharge lamps (Sunlight Supply, Woodland, WA). All accessions were evaluated for fruit and plant type.

Fourteen accessions with the best horticultural qualities in the group (earlier maturity, higher fruit yield and quality, compact plants) were grown at the OSU Vegetable Research Farm, Corvallis, OR, during the summer of 2007 in the S2 generation. ‘Gold Nugget’, ‘Siletz’, and LA1996, a high flavonoid introgression line that expresses the *Anthocyanin fruit tomato* (*Aft*) gene (Jones et al., 2003), were included as standards for low, medium, and high-phenolic tomato lines, respectively. Three replications of five plants each were arranged in a randomized complete block design. Plants were transplanted 21 May; field growth conditions were as described in Mes et al. (2008). Fruit samples for phenolic analysis (one ≈500-g sample per plot) were harvested as fruit reached the red stage of maturity (as defined by Yamaguchi, 1983) in mid-August. Because samples were collected by weight, a variable number of fruit was collected per plot depending on the average fruit size; however, the sample size was based on a minimum of three fruit per plot. Based on the results of the field trial, the five accessions with the best horticultural qualities and the highest phenolic content

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(as measured by F-C; see Folin-Ciocalteu analysis subsequently) were grown under greenhouse conditions in the S3 generation.

Three replicates of one plant each were grown under the same greenhouse conditions as before and evaluated for phenolics content using HPLC. 'Legend', 'Gold Nugget', and LA1996 were used as standards in the second greenhouse evaluation. 'Legend' and 'Siletz' have very similar size and phenolic profiles and 'Legend' served as a substitute standard for 'Siletz'.

Samples and chemicals. Fruit were cut in half lengthwise, and seeds, placenta, and columella tissue were removed. The remaining pericarp and epidermal tissue were ground with liquid nitrogen to a fine powder using a mortar and pestle and weighed portions of this powder were used for extractions. For field samples, 70 to 150 mg of the tomato powder was extracted in 300 μ L of acidified methanol (1% HCl) overnight at 4 °C in 1.5 mL Eppendorf tubes. Extractions were brought to 500 μ L with nanopure H₂O, vortexed with 500 μ L chloroform, and centrifuged at 13,000 rpm for 10 min. The aqueous supernatant was removed and stored at -20 °C for further analysis. The greenhouse-grown samples were processed using a scaled-up version of this protocol (1 g of powdered tissue was extracted in 5 mL 60:40 1% HCl methanol:water and 5 mL chloroform in 15-mL glass tubes). Gallic acid, rutin, and 5-caffeoylquinic acid purchased from Sigma Chemical Corp (St. Louis, MO) were used as standards for F-C and HPLC.

Folin-Ciocalteu analysis. F-C assays were performed according to the method of Singleton and Rossi (1965) and Waterhouse (2006) with modifications. F-C reactions were performed on a microscale: each reaction contained 198 μ L water, 12.5 μ L F-C reagent (Sigma), 2.5 μ L of each sample or gallic acid standard, and 37.5 μ L sodium carbonate solution. Reactions were incubated for 1 h at

room temperature, and absorbance at 765 nm was read using a SPECTRAmax microplate spectrophotometer (Molecular Devices, Sunnyvale, CA).

Liquid Chromatography-Diode Array Detection and Liquid Chromatography-Mass Spectroscopy analysis. HPLC analysis of greenhouse-grown fruit was performed on three samples from different plants. Columns and equipment used were as for Mes et al. (2008). The separation gradient and buffers were as described by Moco et al. (2006). A Hewlett-Packard (Palo Alto, CA) 1050 series autosampler and pump coupled to a HP 1040M diode array detector detection system was used. An ES Industries (West Berlin, NJ) reversed-phase LiChrospher RP18 end-capped column (25 cm $\times$ 4.6 mm, 5 μ m particle size) was used with a guard column of the same material. The separation gradient and buffers followed that of the tomato metabolite database (Moco et al., 2006). Solvent A was formic acid:water (1:1000). Solvent B was formic acid:acetonitrile (1:1000). The flow rate was 1 mL $\cdot$ min⁻¹, and the injection volume was 40 μ L. The elution conditions were as follows: 0 to 45 min, linear gradient from 5% B to 35% B; 45 to 50 min, isocratic at 85% B to wash the column; and 50 to 60 min, isocratic at 5% B to equilibrate the column. Peak spectra were monitored at 280 nm. Ultraviolet spectra were recorded from 200 to 600 nm in 0.5-nm intervals. HPLC data were analyzed using Agilent ChemStation Rev.A.09.01 (Santa Clara, CA). Compounds were quantified as equivalents to chlorogenic acid or rutin using a standard curve and summarized according to compound classes. Standards were used in the range of 25 to 250 mg $\cdot$ L⁻¹ for rutin and 25 to 500 mg $\cdot$ L⁻¹ for chlorogenic acid. Liquid chromatography-mass spectroscopy (LC-MS) was performed using the same column, buffers, and elution conditions on a Shimadzu LC-20-AD chromatography system (Shimadzu,

Columbia, MD) coupled to an ABI 3200 QTRAP LC/MS/MS (Applied Biosystems, Foster City, CA) triple quadrupole mass spectrometer equipped with a Turbo V ionization source.

Statistical analysis. SAS software (Cary, NC) was used for analysis of variance and means separations using Fisher's least significant difference.

Results

A wide range of fruit and plant types were observed among *S. l.* var. *cerasiforme* accessions. The vast majority of *S. l.* var. *cerasiforme* accessions evaluated had extremely late maturity (greater than 120 d) or low yield (measured as the quantity of fruit relative to vegetation) relative to cultivated tomatoes. Because tomato is a facultative short-day plant (Binchy and Morgan, 1970) that flowers best under a photoperiod of 12 h or less, the growth conditions provided (16 h of supplemental lighting) may have inhibited flowering in some accessions. Other undesirable qualities for our objectives included inflorescences which resumed vegetative growth, mealy fruit, seedy fruit, thick-skinned fruit, pale fruit color, and larger fruit (as a result of the inverse correlation between fruit size and phenolic content). Accessions were evaluated for these characters and the 14 best accessions based on a combination of these criteria were selected for further evaluation in a replicated field trial. In many cases, early-maturing accessions were low-yielding, or high-yielding accessions had poor fruit quality, so the final decision was made subjectively.

The 14 selected accessions were evaluated further for horticultural quality (including flavor) in the field trial (Table 1). Fungal diseases including powdery mildew (*Oidium lycopersicum*) and gray mold (*Botrytis cinerea*) were a problem in some accessions and strong

Table 1. Phenolic content of fruit extracts, average fruit weights, and field notes for *S. l.* var. *cerasiforme* accessions and cultivated tomatoes from 2007 field trial.^a

Entry	Mean GAE	SE	Field notes	Avg fruit wt (g)
LA1712	81.8 _a	± 15.4	Very acidic; poor fungal disease resistance; low to medium yield; late maturity	5.0
LA1455	75.5 _{ab}	± 8.7	Sweet fruit; upright growth habit; thick skin; good yield and yield architecture; flavor ok; fruit with glandular trichomes	3.3
LA2633	73.0 _{abc}	± 3.3	Hard skin, very sweet, high solids; later maturity, good yield, long fruit trusses	2.3
LA1668	68.2 _{abcd}	± 4.0	High yield; upright growth habit; sweet, good quality fruit; thin skin	4.0
LA2632	67.5 _{abcde}	± 4.4	Very small pubescent fruit; poor flavor (bland); firm pericarp	2.1
LA1620	63.6 _{abcdef}	± 6.5	Good yield and fungal disease resistance; relatively good fruit quality, excessive gel on seeds, earlier than LA1549	5.9
LA1549	63.2 _{abcdefg}	± 3.0	Good yield and fungal disease resistance; firm fruit, flavor ok	4.3
LA2135	60.4 _{abcdefg}	± 9.0	Early, poor resistance to fungal diseases; medium to poor fruit quality; medium size, lower yielding	13.3
LA1338	57.2 _{bcddefg}	± 17.3	Early, very dark red fruit; very poor fungal disease resistance; horrible flavor (possibly resulting from fungal infection?)	7.1
LA2626	51.9 _{cdefg}	± 7.2	Bad taste (astringent or causing a prickly sensation in throat); poor fungal disease resistance; good yield, early maturing	6.0
LA1290	45.4 _{defg}	± 0.8	Larger fruit, watery flavor; good yield; variable fruit shape	14.2
LA2308	44.2 _{efg}	± 1.7	Large fruit; medium to good yield; bitter flavor, very astringent	23.6
Afi (LA1996)	48.9 _{defg}	± 8.1	Anthocyanin fruit, contains a gene from <i>S. chilense</i> resulting in anthocyanin production in fruit; used as a standard for a large fruited, high-phenolic tomato	90.5
Gold Nugget	42.3 _{fg}	± 10.6	A yellow-fruited cherry tomato developed for use in the Pacific Northwest, used as a standard for a small-fruited tomato	11.8
Siletz	40.0 _g	± 5.2	A red-fruited tomato developed for use in the Pacific Northwest, used as a standard for a large-fruited tomato	204.6

^aPhenolics content of fruit extracts was measured by Folin-Ciocalteu assay and expressed as gallic acid equivalents (GAE). Means followed by the same letter are not significantly different as measured by Fisher's least significant difference ($P \leq 0.05$). Fruit weights are the average of 10 ripe fruit.

genotype-dependent responses were observed. There was no correlation between phenolic content of the fruit and fungal disease resistance (Table 1). Some of the best accessions in terms of horticultural quality (for a cherry tomato) were LA1620, LA1549, LA1668, LA1512, LA2633, and LA1455.

Total phenolic content of fruit extracts from field-grown material are presented in Table 1. Five accessions (LA1455, LA1668, LA1712, LA2632, and LA2633) had significantly higher fruit phenolics than cultivars as measured by the F-C assay (total phenolics F-C) (Table 1). Only LA1455, LA1712, and LA2633 had significantly higher total phenolics than LA1996 (Table 1). There was a modest but statistically significant negative correlation between total phenolics (F-C) and fruit weight (Pearson's correlation coefficient = -0.49 , $P = 0.046$). Five accessions were selected for further evaluation in the greenhouse (LA1455, LA1549, LA1620, LA1712, and LA2633). The decision of which accessions to use for further evaluation was based on a combination of their F-C total phenolic scores and their horticultural quality (Table 1).

HPLC-photodiode array (PDA) information for greenhouse-grown material (Fig. 1) was based on three replicates (each replicate consisted of ripe fruit tissue from an individual greenhouse-grown plant). LA2633 was notable for having levels of quercetin and kaempferol derivatives that were significantly higher than cultivars and many other *S. l. var. cerasiforme* accessions. Most *S. l. var. cerasiforme* accessions had significantly higher levels of mono-, di-, and triacylquinic acids than cultivars.

Peak assignments (Figs. 2 and 3; Table 2) were made using a combination of peak retention times, ultraviolet-spectra, and mass spectra. Mass spectra and ultraviolet-spectra were acquired on separate instruments. Mass spectra were only acquired for the genotypes Legend and LA2633. Retention times for major tomato phenolic compounds (e.g., chlorogenic acid, rutin, and naringenin chalcone) were ≈ 2 min later on the HPLC-MS system than on the HPLC-PDA system, but the chromatograms could still be aligned fairly well (Figs. 2 and 3). Twelve peaks were positively identified using a combination of ultraviolet- and accurate mass spectra (Table 2). HPLC-PDA chromatograms (Fig. 3) revealed that *S. l. var. cerasiforme* accessions contained numerous compounds that were not detected or detected in only trace quantities from cultivars (e.g., Peaks 8, 9, and 10). Phenolic content was summarized by compound class (caffeoylquinic acids, Peak 2; diacylquinic acids, Peak 9; quercetin derivatives, Peaks 3 and 6; rutin, Peak 7) and quantitated using a standard curve from chlorogenic acid (caffeoylquinic acids) or rutin (quercetin derivatives) based on peak identifications in Table 2 and/or peak assignments to compound class (Fig. 1). Standard curves were linear over the range sampled ($R^2 \geq 0.9816$).

Discussion

Tomato is a model species for the study of fruit development and there has been a recent

Fig. 1. Phenolic content (mg/100 g fresh weight, measured by high-performance liquid chromatography-photodiode array) of *S. l. var. cerasiforme* accessions. Values are the mean of three biological replicates; error bars = 1 SE. Means sharing a letter (within a series of phenolic compounds) are not significantly different, Fisher's least significant difference, $P \leq 0.05$. Series abbreviations: RUT = rutin; QUE = quercetin derivative other than rutin; CQA = caffeoylquinic acid isomers; DI = dicaffeoylquinic acid isomers.

explosion in metabolomics research related to analysis of phenolic compounds in tomato (Iijima et al., 2008; Mintz-Oron et al., 2008; Moco et al., 2006, 2007). Many compounds present only in trace amounts in cultivated tomato were present at higher levels in the *S. l. var. cerasiforme* accessions evaluated here, including quercetin 3- α -apiosyl-6- α -rhamnosylglucoside (Butelli et al., 2008; Slimstad et al., 2008), a quercetin dihexose-deoxyhexose (Iijima et al., 2008; Moco et al., 2007), feruloylquinic acid (Moco et al., 2006), dicaffeoylquinic acids (Luo et al., 2008; Moco et al., 2006), and tricaffeoylquinic acids (Luo et al., 2008; Moco et al., 2006). We also found two putative isomers of feruloylquinic acid ($[M-H]^- = 367.0$) in which only one has been previously reported in tomato (Moco et al., 2006).

Many of the 100 *S. l. var. cerasiforme* accessions that were evaluated in the greenhouse had very late maturity (over 120 d) or low yields resulting from excessive vegetative growth and were not evaluated for phenolic content in the field or otherwise. Field evaluation revealed that some accessions suffered

from susceptibility to fungal diseases, poor flavor, or other defects (Table 1). LA1712, LA1455, LA2633, and LA1549 were accessions with better horticultural quality and higher phenolic content over multiple environments. There was no correlation between resistance to fungal diseases and fruit phenolic content (Table 1). However, leaf phenolic content might be expected to play a more important role in influencing disease resistance. Leaf phenolic content of the 14 accessions in the field trial was measured by F-C and ranged from 322 GAE to 979 GAE but showed poor correlation with levels of fungal disease resistance (data not shown).

HPLC-PDA chromatograms of these accessions revealed important differences in their phenolic profiles (Fig. 2). LA1712 (from Pejibaye, Costa Rica) was notable for having large amounts of di- and tricaffeoylquinic acids (Peaks 9 and 10). LA1712 possessed large amounts of a phenolic compound that was not positively identified (the large peak in between Peaks 7 and 9). This peak had a retention time of 32.44 min and an ultraviolet_{max}

Fig. 2. High-performance liquid chromatography–photodiode array chromatograms (280 nm) of selected accessions in this study from greenhouse-grown fruit. For peak assignments, see Table 2. All chromatograms to the same scale.

Fig. 3. Total ion chromatograms of LA2633 and 'Legend' from liquid chromatography–mass spectroscopy analysis. Peak assignments are as for Figure 2 and Table 2.

of 284 nm with a shoulder at 326 nm, a spectrum that could be consistent with a chalcone derivative or a coumaric acid derivative. This compound was one of the predominant phenolics in LA1712 but was present in only trace

amounts in other accessions. Because mass spectra were only obtained for the genotypes LA2633 and 'Legend', mass spectra for this peak were not available. Because this peak was not positively identified, it was not assigned

a peak number (Figs. 2 and 3; Table 2). LA1712 had poor fungal disease resistance and very acidic flavor, however. LA1455 (from Nuevo Leon, Mexico) had very high levels of 4-caffeoylquinic acid (chlorogenic acid). LA1455 also had good (upright) plant architecture and yield and small, sweet, glandular fruit. LA2633 (from Cusco, Peru) had high levels of chlorogenic acid (Peak 2), kaempferol and quercetin derivatives (Peaks 3, 6, 7, and 8), and di- and triacylquinic acids (Peaks 9 and 10). LA2633 had hard skin, sweet fruit with high solids, good yield, and long fruit trusses. LA1549 (from Pasco, Peru) had moderately high levels of chlorogenic acid (Peak 2), quercetin derivatives (Peaks 3, 6, and 7), and di- and tricaffeoylquinic acids (Peaks 9 and 10).

The levels of phenolic compounds found in tomatoes or other fruits vary greatly depending on the sample preparation method, tissue(s) sampled, genotype, and growing conditions (Robbins, 2003). Among cultivated tomatoes, tissue type and maturity stage have major effects on the quantity and profile of compounds (Moco et al., 2007). According to the USDA Database for the Flavonoid Content of Selected Foods, Release 2.1 (United States Department of Agriculture, 2007), the year-round average naringenin and quercetin content of red tomatoes is 0.68 and 0.58 mg/100 g FW, respectively; whereas for cherry tomatoes, these values are 3.19 and 2.76 mg/100 g FW, respectively. These values are based on the entire edible portion of the fruit. In this study, seeds, gel, and columellar tissue were excluded because the inclusion of liquid nitrogen ground seeds in the extraction might affect the fruit phenolic profile in ways that are biologically unrealistic as far as human consumption is concerned. Another reason for excluding these tissues was to avoid the dilution of minor compounds below the limit of detection without concentration of the samples, which can cause degradation. We report values four to eight times those of the USDA for cultivated tomatoes in the tissue sampled here (pericarp and epidermis). This value is reasonable given that the seeds, columella, gel, and placental tissues constitute a significant portion of the weight of a fresh tomato and the phenolics are concentrated in the outer layers of the pericarp and epidermis. Recently, flavonoid concentrations of up to 200 mg/100 g FW have been reported for tomato peel (Giuntini et al., 2008; Torres et al., 2005). Thus, we believe that the high concentration of quercetin and other phenolics reported here for some small-fruited, wild accessions (up to 70 mg/100 g FW quercetin in LA2633 under greenhouse conditions) is relatively accurate.

In general, *S. l.* var. *cerasiforme* accessions did not have particularly high levels of naringenin (Peak 11) or naringenin chalcone (Peak 12), which have sometimes been reported to be the predominant flavonoids in tomato. In fact, the highest levels of these compounds were found in the cultivar Gold Nugget in our study (Fig. 2). We mainly observed naringenin chalcone rather than naringenin. This is in agreement with the observation of Sliemstad

Table 2. Metabolites identified by high-performance liquid chromatography–photodiode array (HPLC-PDA) and liquid chromatography–mass spectroscopy.^z

Peak	RT	Mass	ID	Ultraviolet-Vis	Reference
1	15.17	341.0	Caffeic acid hexose	328, 298sh	Moco et al., 2007
2	17.08	353.5	4- and 5-caffeoylquinic acid	326, 298sh; 320, 290sh	Moco et al., 2007
3	18.25	771.3	Quercetin dihexose-deoxyhexose (quercetin 3-o-rutinoside-7-o-glucoside)	256, 352	Iijima et al., 2008; Moco et al., 2007
4	21.48	367.0	Feruloylquinic acid isomer	328, 298sh	Clifford et al., 2003; Kammerer et al., 2004; Moco et al., 2006
5	25.23	367.0	Feruloylquinic acid isomer	326	Clifford et al., 2003; Moco et al., 2006
6	25.74	741.3	Quercetin 3-2'' apiosyl-6'' rhamnosylglucoside, rutin pentoside, Quercetin hexose-deoxyhexose pentose	256, 350	Giuntini et al., 2008; Iijima et al., 2008; Slimestad et al., 2008
7	28.23	609.2	Rutin	256, 354	Slimestad et al., 2008; many others
8	31.31	593.2	Kaempferol 3-6'' rhamnosylglucoside	264, 344	Iijima et al., 2008; Moco et al., 2007; Slimestad et al., 2008
9	33.98	515.3	Dicafeoylquinic acid	326, 298sh	Clifford et al., 2003; Moco et al., 2006
10	42.99	677.3	Tricafeoylquinic acid	330, 294sh	Clifford et al., 2003; Moco et al., 2006
11	47.74	NA	Naringenin	288, 344sh	Slimestad et al., 2008; many others
12	48.5	271.0	Naringenin chalcone	366	Slimestad et al., 2008; many others

^zPeaks (identified in Figs. 2 and 3), retention time (RT) under HPLC-PDA conditions, mass ([M-H]⁺), identification (ID), observed ultraviolet_{max}, and references from the literature.

NA = not available; sh = shoulder of ultraviolet_{max}.

et al. (2008) that naringenin chalcone is the native compound. According to Slimestad et al. (2008), reports of naringenin as the predominant compound are the result of the spontaneous isomerization of naringenin chalcone into naringenin. The two compounds have very different ultraviolet maxima, and because standards for naringenin chalcone were not available to us, we did not estimate naringenin chalcone levels.

Hanson et al. (2004) reported total phenolics as measured by the F-C method in tomato in the range of 60 to 90 mg/100 g FW for cultivars and 150 mg/100 g FW for small-fruited *S. pimpinellifolium* accessions. Hernández et al. (2007) reported lower values in the range of 10 to 30 mg/100 g FW for cultivated tomatoes. These differences could be the result of the sample preparation method (fresh versus frozen puree, respectively), but differences in the standard curves are another possible explanation. We report values intermediate between the two studies (40 mg/100 g FW).

Smaller tomato fruit also have higher phenolic concentrations as a result of their increased surface area:volume ratio. There were several indications that the high phenolic content observed in *S. l.* var. *cerasiforme* accessions was not the result of small fruit size alone, however. We found a significant correlation between fruit size and phenolics content, but fruit size was not strictly correlated with phenolic content, especially among accessions of *S. l.* var. *cerasiforme* (e.g., LA1512 had small fruit but only modest phenolic levels).

Several *S. l.* var. *cerasiforme* accessions had levels of di- and triacylquinic acids that were significantly higher than cultivated tomatoes (Fig. 1), making the *S. l.* var. *cerasiforme* accessions evaluated here a good source for this trait. MicroTom cultivars transformed with *AtMYB12* produced di- and triacylquinic acids at a concentration of 4 to 6 mg·g⁻¹ dry weight (Luo et al., 2008), a level similar to that observed for high-phenolic *S. l.* var. *cerasiforme* accessions in this study (4 to 7 mg/100 g FW). The genetic basis of this trait therefore seems more likely to be the result of a

difference in regulation of the pathway leading to these compounds rather than a lack of the necessary structural genes in the cultivated tomato.

This study provides the basis for a more extensive candidate gene analysis using classical mutants, mapped expressed sequence tags, and the tomato genome sequence. Because the Tanksley core set has been genotyped extensively, this information could be to facilitate introgression of high phenolic traits. S3 plants of LA1455, LA1549, LA1620, LA1712, and LA2633 were crossed to high anthocyanin (*AftAft/atvatv*) and high flavonol (*AftAft/atvatv/awaw*) tomato lines with the objective of developing tomato lines that have elevated fruit phenolic levels or unique phenolic profiles. Some of these populations could also be used for mapping genes related to the phenolic biosynthetic pathway. LA2633 exhibits a high degree of DNA marker polymorphism (Boches, 2009) as well as elevated levels of a wide range of polyphenolics, making it an ideal candidate for developing mapping populations. In contrast, *S. l.* var. *cerasiforme* accessions with high phenolics and a lower proportion of wild species alleles such as LA1712 (Boches, 2009) might make better candidates for the introducing the trait into cultivars to reduce linkage drag to unfavorable alleles for other traits.

In conclusion, screening of a genotypically characterized *S. l.* var. *cerasiforme* core collection for accessions with better horticultural quality and high phenolic content identified several accessions with elevated fruit phenolic content and unique fruit phenolic profiles. These accessions represent a useful resource for developing high-phenolic tomato germplasm or identifying genes associated with phenylpropanoid metabolism.

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