

Exploration of Three *Solanum* Species for Improvement of Antioxidant Traits in Tomato

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Abstract. Wild tomato species have been widely used for improvement of tomato disease resistance but have not been extensively explored for health-related traits. In this work, three interspecific populations derived from backcrosses between cultivated tomato and *Solanum pimpinellifolium* (LA1589), *S. habrochaites* (LA1223), and *S. peruvianum* (LA2172) were analyzed for water-soluble antioxidant activity, phenolic content, vitamin C content, and basic agronomic traits including fruit weight, shape, and color. The wild species accessions significantly exceeded *S. lycopersicum* for all three antioxidant traits with only one exception: vitamin C content in *S. habrochaites* LA1223. Several populations and traits showed transgressive segregation indicating that the backcross populations contained individuals with allele combinations that allowed antioxidant activity/content to exceed that of both parents. The *S. habrochaites* LA1223 population provided the best starting material for improvement of water-soluble antioxidant activity and phenolics content with 20% and 15% of the population, respectively, significantly exceeding the parental values for these traits. Moreover, the *S. habrochaites* population contained individuals that had nearly 2-fold more water-soluble antioxidant activity and phenolic content than cultivated tomato. The *S. peruvianum* LA2172 population was best for improvement of vitamin C content with 3-fold variation for the trait and individuals, which had twice as much vitamin C as cultivated tomato.

Tomato, *Solanum lycopersicum* (syn. *Lycopersicon esculentum*), is a member of the Solanaceae, a plant family of more than 3000 species including the economically important crops potato, eggplant, pepper, and tobacco. Tomato ranks second after potato as the most consumed vegetable of this family. Currently, tomato is grown in almost all countries worldwide. However, the origins of tomato are believed to be in western South America: Chile, Bolivia, Ecuador, and the coastal region of Peru (Razdan and Mattoo, 2007) where wild tomato species, including *S. pimpinellifolium*, *S. pennellii*, *S. habrochaites*, *S. peruvianum*, *S. chmielewskii*, and *S. chilense*, are still found (Peralta et al., 2006).

Wild tomato species are sources of both desired and undesired traits. *S. peruvianum* (syn. *L. peruvianum*) is genetically and morphologically the most diverse of the wild

tomatoes (Peralta et al., 2005). It is so polymorphic that *S. peruvianum* was divided into varieties including dentatum (Dunal) and humifusum (Müller). *S. peruvianum* has green fruits, is typically self-incompatible, and allogamous. This species is also known for its virus, bacteria, fungi, aphid, and nematode resistances and has been widely used to introduce these traits into cultivated tomato (Robertson and Labate, 2007). *S. habrochaites* (syn. *L. hirsutum*) is another wild species used in the improvement and breeding of tomatoes. It has green fruits like *S. peruvianum* and is self-incompatible. *S. habrochaites* has a wide range of biotic and abiotic stress tolerances such as bacterial (Francis et al., 2001; Kabelka et al., 2002), fungal (Zhang et al., 2003), viral (Momotaz et al., 2005), insect (Musetti and Neal, 1997; Snyder et al., 1993), and cold (Veneema et al., 2000) resistances. Many of these resistances have been incorporated into cultivated tomato by backcross breeding (Hajjar and Hodgkin, 2007; Kallou, 1991). *S. pimpinellifolium* (syn. *L. pimpinellifolium*) is a self-compatible tomato with red fruit. It is autogamous and facultatively allogamous. It is closely related to *S. lycopersicum* (Peralta and Spooner, 2007) and has been used in breeding to improve fruit color and quality (Causse et al., 2007). To date, most of the work using wild species for improvement of tomato has focused on biotic stress resistance. In comparison, only superficial and

partial assessment of genetic variation for abiotic stress and nutritional factors such as antioxidant compound content has been made in wild tomato species (Causse et al., 2007).

Tomato is rich in various vitamins, antioxidants, and secondary metabolites (Adalid et al., 2004). The most abundant of these molecules are vitamin C, lycopene, β -carotene, and phenolics. These compounds are all known antioxidants, which are reported to be important for human health as well as a plant defense to abiotic and biotic stresses (Khachik et al., 2002). However, during domestication and later breeding, farmers and plant breeders focused on agronomically important traits of tomato such as fruit weight, color, shape, and disease resistance. Now that we know the genetic mechanisms of some of these phenotypic traits, it is possible to breed agricultural plants with more precision for what is needed. Often the best source for improvement of a crop plant is its wild species (Tanksley and McCouch, 1997). Wild species retain allelic (trait) diversity, which has been lost during domestication and breeding.

In this work, antioxidant and agronomic traits of three wild tomato species accessions, *S. peruvianum* LA2172, *S. habrochaites* LA1223, and *S. pimpinellifolium* LA1589, were compared with cultivated tomato. Antioxidant traits are of interest to consumers and plant breeders for their health-related contributions as well as their importance in plant resistance to biotic and abiotic stresses. The main aim of this work was to determine the potential of these wild species accessions for improvement of different traits. These results can be used in designing a breeding program to improve elite tomato lines for antioxidant characteristics.

Materials and Methods

The *S. peruvianum* advanced backcross BC₂F₂ population was obtained by crossing *S. lycopersicum* (TA496) as the cultivated recurrent parent with *S. peruvianum* (LA2172) as the wild donor parent. TA496 is the processing tomato line E6203 with the addition of tomato mosaic virus resistance. Because crossability barriers between *S. peruvianum* LA2172 and *S. lycopersicum* prevent normal development of F₁ seeds, ovule culture was used to rescue the hybrids as described in Doganlar et al. (1997). F₁ hybrids were then backcrossed to TA496 for two generations with no further need for ovule culture. Self-pollination of the BC₂F₁ families resulted in the BC₂F₂ families that were used in this work. Overall, the Peruvianum population contained 88 lines.

For the Habrochaites studies, a BC₂F₂ population was developed by crossing *S. lycopersicum* (TA1166) as the recurrent parent and *S. habrochaites* (LA1223) as the donor parent. TA1166 is a fresh-market inbred line (5TSS802 from Harris Moran). Population development was the same as for the Peruvianum population except that ovule culture was not necessary. The Habrochaites population contained 151 lines.

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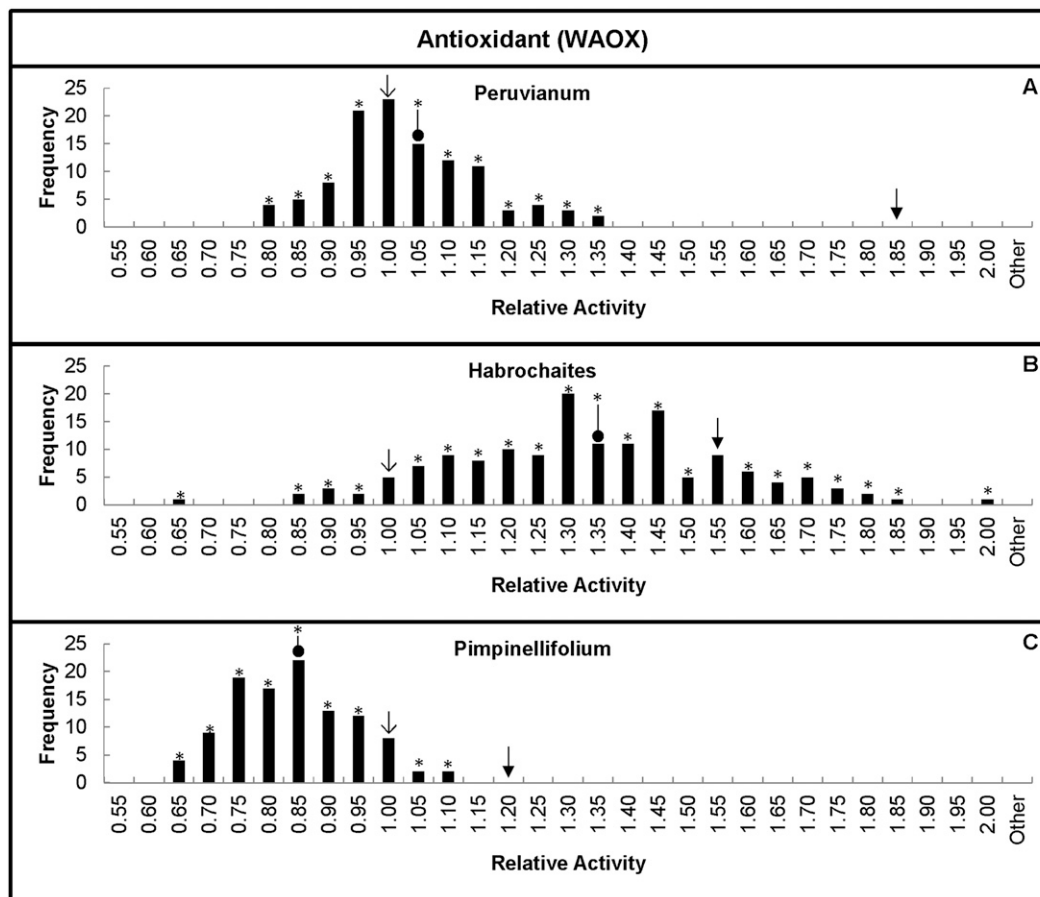


Fig. 1. Water-soluble antioxidant activity for (A) *S. peruvianum* LA2172, (B) *S. habrochaites* LA1223, and (C) *S. pimpinellifolium* LA1589-derived populations relative to *S. lycopersicum*. Cultivated parent, wild-type parent, and mean of the population are shown with open, closed, and spherical arrows, respectively. Asterisks indicate bins with real values significantly different from *S. lycopersicum* as determined with Dunnett's method for means comparison ($P \leq 0.05$).

The *Pimpinellifolium* studies were performed with a BC₂F₉ population that was developed by crossing recurrent parent *S. lycopersicum* (TA209) and *S. pimpinellifolium* (LA1589), the wild donor parent. TA209 is processing line E6203 without virus resistance. One F₁ hybrid was selected from the interspecific cross and backcrossed to TA209. A total of 27 BC₁ plants were then selected based on presence of the *sp* locus on chromosome 6 so that all progeny plants had determinate growth. These selected individuals were backcrossed to TA209 to produce the BC₂ population. BC₂F₁ individuals were then selfed for eight generations to produce the BC₂F₉ population. The *Pimpinellifolium* population contained 109 lines.

The *Peruvianum* population and parental lines were grown in the field at MultiTarim Seed Co., Antalya, Turkey, from late March to June 2006. The *Habrochaites* population and parents were also field-grown in Antalya from early April to July 2007. During the growing season, mean minimum temperature ranged from 11 to 22 °C, whereas mean maximum temperature ranged from 21 to 34 °C. Ten plants of each population and parental line were grown and were transplanted in double rows with 140-cm wide rows and 50-cm narrow row spacing. Double-row spacing is common in coastal regions and

provides shade for fruit. Plants were spaced at 40 cm within rows. Soil was loamy and well-drained and fertilized with 15:15:15 fertilizer and 50 t composted manure per hectare before transplanting. Plants were drip-irrigated with fertigation (1.4 dS·m⁻¹ electrical conductivity value) during the growing season (1:2:1 until first fruit set, 2:1:1 until first ripe fruit, 1:1:2 after first ripe fruit).

The *Pimpinellifolium* population was grown in the field at the Aegean Agricultural Research Institute, Izmir, Turkey, from late April to early Aug. 2006. During the growing season, mean minimum temperature ranged from 11 to 22 °C, whereas mean maximum temperature ranged from 21 to 33 °C. Ten plants of each population and parental line were grown in single rows with 140 cm between rows and 40 cm between plants within rows. The soil at the Izmir site was sandy loam and was fertilized with 30 kg·d⁻¹ potassium sulfate, 25 kg·d⁻¹ di-ammonium phosphate, and 10 kg·d⁻¹ ammonium sulfate before transplanting. Plants were drip-irrigated with 30 kg·d⁻¹ ammonium nitrate and 10 kg·d⁻¹ potassium nitrate fertilizer. Plants were treated three times with 2% calcium nitrate to prevent blossom-end rot during fruit development. At both locations, fruit were harvested at the normal ripe market stage with samples bulked from the different plants for each line.

Total water-soluble antioxidant (WAOX) activity, total vitamin C (VITC) content, and total phenolic (PHE) content were determined for each line in each population. Ten ripe tomato fruits were selected from the harvested fruit. Care was taken to ensure that the selected tomatoes were of average size, color, and ripeness. The tomatoes were diced, mixed, and stored at -20 °C. All analyses were done within 6 months. For analysis, 100 g ripe tomato fruits were homogenized in 200 mL ice-cold distilled water for 2 min. For each 10 mL of mixture, 15 mL of ice-cold distilled water was added to the homogenate. The mixture was filtered using nylon cloth and filtrate was centrifuged at 3000 g at 4 °C for 20 min. The supernatant was prepared for antioxidant analyses by filtration through four layers of nylon cloth. WAOX measurements were performed as described by Re et al. (1999) using the ABTS cation decolorization assay and Trolox as a standard. WAOX activity was expressed as μmol Trolox/kg fresh weight. VITC was measured by the AOAC 967.21 titrimetric method as described by Augustin (1994) using commercial L-ascorbic acid as control and expressed as mg·kg⁻¹ fresh weight. PHE was measured with the method developed by Singleton and Rossi (1965) and expressed as gallic acid equivalents (mg·kg⁻¹ fresh weight). Each

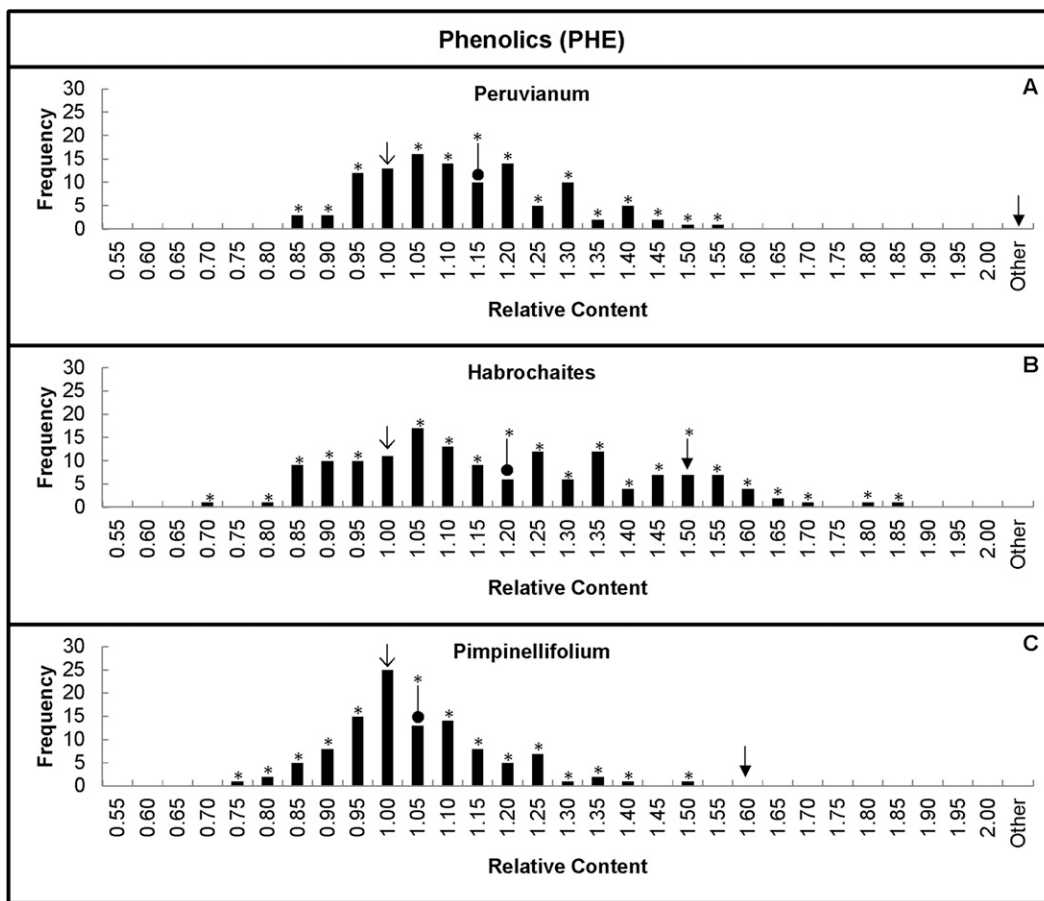


Fig. 2. Phenolic content for (A) *S. peruvianum* LA2172, (B) *S. habrochaites* LA1223, and (C) *S. pimpinellifolium* LA1589-derived populations relative to *S. lycopersicum*. Cultivated parent, wild-type parent, and mean of the population are shown with open, closed, and spherical arrows, respectively. Asterisks indicate bins with real values significantly different from *S. lycopersicum* as determined with Dunnett's method for means comparison ($P \leq 0.05$).

measurement was performed with three replicates. Averages of the replicates were taken and used for statistical analysis.

Fruit weight (FW) was determined by weighing at least 10 fruits per line. Fruit shape (FS), firmness (FIRM), external (EC) and internal (IC) color were scored by visual inspection of at least 10 ripe fruits per line. A scale of 1 to 5 was used for scoring with a different meaning for each trait (FS: 1 = round, 5 = elongated; EC: 1 = yellow or orange, 5 = dark red; FIRM was determined by manual squeezing of fruit (1 = very soft, 5 = very firm). IC was determined by examination of at least 10 transversely cut fruits. IC was scored with the same scale as EC.

Summary statistics of the data for each species were calculated. Means for quantitative data were compared with Student's *t* test with a *P* value threshold of 0.05. All population values were then divided by the *S. lycopersicum* parental values to obtain normalized contents. Such normalization is a way to minimize variability in metabolite content resulting from environmental conditions (Perez-Fons et al., 2014). Histograms for antioxidant and agronomic traits measurements were drawn using the normalized data. Significant differences among bins in the histograms were determined by using the raw data for each bin and Dunnett's method

to compare each bin to the *S. lycopersicum* bin ($P \leq 0.05$). Correlation tables were constructed using the raw data obtained from the measurements of fruits of each *Solanum* species. All statistical analysis was performed with the JMP 7.0 statistical program (SAS Institute, Cary, NC).

Results

Total water-soluble antioxidant activity. WAOX activity of the *S. peruvianum* accession ($8764 \pm 110 \mu\text{mol Trolox/kg}$) was highest followed by the *S. pimpinellifolium* ($6989 \pm 249 \mu\text{mol Trolox/kg}$) and *S. habrochaites* ($3925 \pm 11 \mu\text{mol Trolox/kg}$) accessions (Fig. 1). For all three species, WAOX activity of the interspecific populations showed continuous variation as expected for a quantitative trait. WAOX activity of *S. peruvianum* fruit was significantly higher than *S. lycopersicum* (closed arrow in histogram) with a 1.8-fold difference in values. The population mean was only slightly higher than the value for *S. lycopersicum*. The WAOX activity of the Peruvianum population showed a 1.8-fold range. In addition, 32% of the population had significantly higher WAOX activity than *S. lycopersicum*. WAOX activity of fruit from the *S. habrochaites* accession was significantly higher (1.5-fold) than the WAOX

activity of *S. lycopersicum*. The WAOX activity of the Habrochaites population showed a 3.2-fold range. The population mean was closer to the wild parental mean than the mean of *S. lycopersicum*. In addition, 91% of the population had significantly higher WAOX activity than *S. lycopersicum*. Moreover, 20% of the population had significantly higher activity than both parents indicating substantial transgressive segregation for this trait. WAOX activity of fruit from the *S. pimpinellifolium* accession was 1.2-fold higher than for *S. lycopersicum*, a significant difference. The WAOX activity of the Pimpinellifolium population showed a 2-fold range. The mean of the population was closer to *S. lycopersicum*. Only 5% of the population had significantly higher activity than *S. lycopersicum*.

Phenolic content. The PHE content of the *S. peruvianum* accession was highest ($1619 \pm 20 \text{ mg}\cdot\text{kg}^{-1}$) followed by the *S. pimpinellifolium* ($844 \pm 48 \text{ mg}\cdot\text{kg}^{-1}$) and *S. habrochaites* ($303 \pm 14 \text{ mg}\cdot\text{kg}^{-1}$) accessions (Fig. 2). PHE contents of the three populations were distributed as expected for a complex trait controlled by more than one gene. PHE content of the *S. peruvianum* accession was 3.2-fold higher than cultivated tomato, a significant difference. There was nearly 2-fold variation in PHE content in the population.

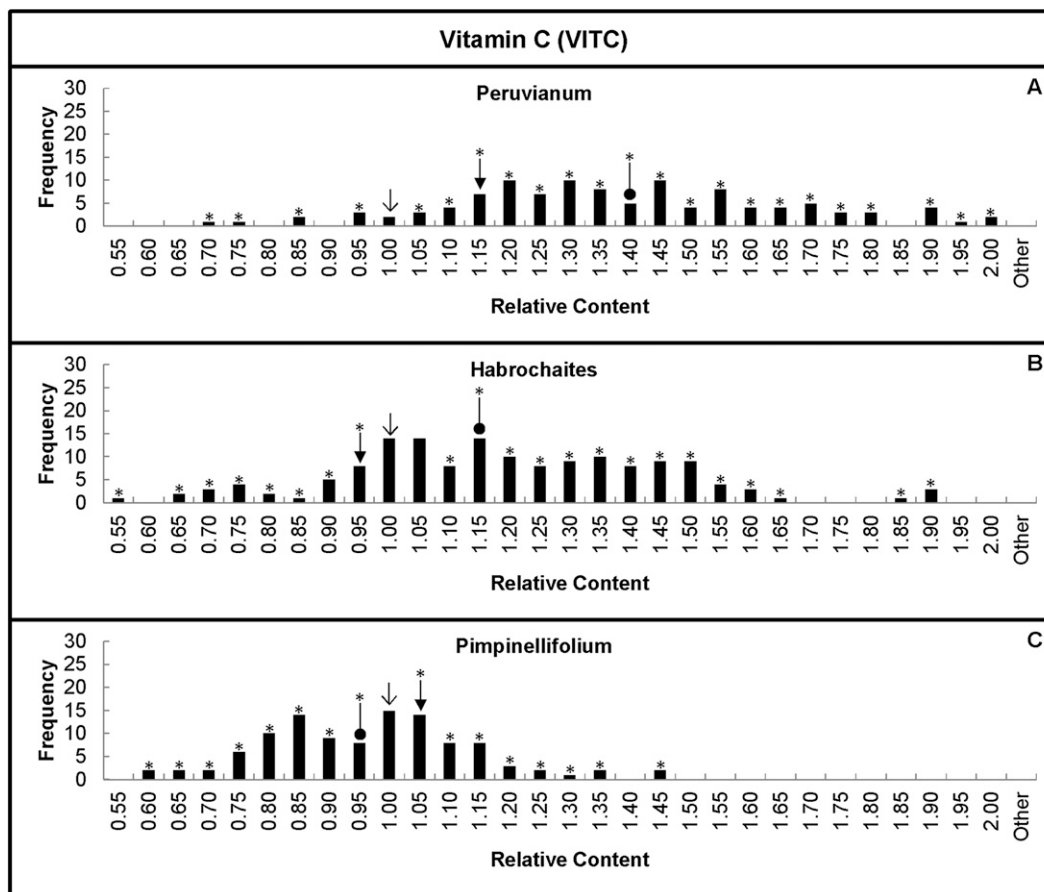


Fig. 3. Vitamin C content for (A) *S. peruvianum* LA2172, (B) *S. habrochaites* LA1223, and (C) *S. pimpinellifolium* LA1589-derived populations relative to *S. lycopersicum*. Cultivated parent, wild-type parent, and mean of the population are shown with open, closed, and spherical arrows, respectively. Asterisks indicate bins with real values significantly different from *S. lycopersicum* as determined with Dunnett's method for means comparison ($P \leq 0.05$).

Approximately 70% of the population had significantly higher PHE values than *S. lycopersicum*; however, no individuals exceeded the wild parent value. The *S. habrochaites* accession had significantly higher (1.5-fold) PHE content than *S. lycopersicum* (Fig. 2). The PHE content of the Habrochaites population showed a 3.2-fold range. The mean value of the PHE content for the population was intermediate between the parental lines. In addition, 15% of the population had significantly higher PHE content than both parental lines as a result of transgressive segregation. The PHE content of the *S. pimpinellifolium* accession was 1.6-fold higher than *S. lycopersicum*, a significant difference. Approximately 48% of the population showed significantly higher values than *S. lycopersicum*.

Vitamin C content. VITC content of the *S. pimpinellifolium* accession was highest ($31.5 \pm 8.0 \text{ mg}\cdot\text{kg}^{-1}$) followed by the *S. peruvianum* ($27.1 \pm 2.0 \text{ mg}\cdot\text{kg}^{-1}$) and *S. habrochaites* ($16.0 \pm 2.0 \text{ mg}\cdot\text{kg}^{-1}$) accessions (Fig. 3). All three populations showed continuous variation as expected. VITC content of the *S. peruvianum* accession was 1.1-fold higher than *S. lycopersicum*, a small but significant difference. In the population, VITC content showed more than 3-fold variation for the trait. In the Peruvianum population, 82% of the progeny had significantly higher VITC content than both parents.

The VITC content of the *S. habrochaites* accession was 0.9-fold lower than the VITC content of *S. lycopersicum*. This was the only antioxidant trait-accession combination for which the wild species had a lower value than the cultivated type. The Habrochaites population showed distinct 4-fold variation. Approximately 80% of the population had significantly higher VITC content than both parents and 70% of the population had higher values than cultivated tomato. VITC content of the *S. pimpinellifolium* accession was only slightly higher than *S. lycopersicum*. The VITC content in the Pimpinellifolium population showed a 2.7-fold range in values. Overall, 37% of the population had significantly higher values than *S. lycopersicum*.

Fruit weight. As expected, cultivated tomato fruits were largest, whereas the three wild species accessions all had significantly smaller fruit (ranging from 1.5 to 6.5 g) than the *S. lycopersicum* accessions. FW of *S. lycopersicum* was 21-fold higher than the *S. peruvianum* accession and there was a 2.5-fold range in values in the population (Fig. 4). There was greater variation for FW in the Habrochaites population. The two parental lines showed extremely different values for FW, a 40-fold difference. There was also great variation in the Pimpinellifolium population, a 4-fold range. Weight for *S. lycopersicum* was 48-fold higher than the *S. pimpinellifolium*

accession. The Pimpinellifolium population was the only one that had individuals that exceeded *S. lycopersicum* for FW.

Fruit shape. FS of the *S. lycopersicum* and *S. peruvianum* accessions were different from each other. The *S. lycopersicum* accession had somewhat elongated fruit and the *S. peruvianum* accession had round fruit. There was good variation ranging between round and elongated types in the Peruvianum population (Supplemental Fig. 1A). Progeny were mostly intermediate between the parents and 21% of the population had rounder fruit than the average. In both *S. habrochaites* and *S. lycopersicum* parental lines, FS was spherical (Supplemental Fig. 1B). However, there was also variation ranging between round and elongated types in the Habrochaites population. Approximately 39% of the population had elongated fruit. For the Pimpinellifolium population, the *S. lycopersicum* accession fruits were somewhat elongated and *S. pimpinellifolium* fruits were round (Supplemental Fig. 1C). For the population, the range was between round and elongated. Approximately 11% of the population showed more elongated fruits than *S. lycopersicum* as a result of transgressive segregation.

Firmness. The *S. peruvianum* accession had firmer fruit than *S. lycopersicum* (Supplemental Fig. 2A). In the Peruvianum population, 30% of the population had firmer fruit

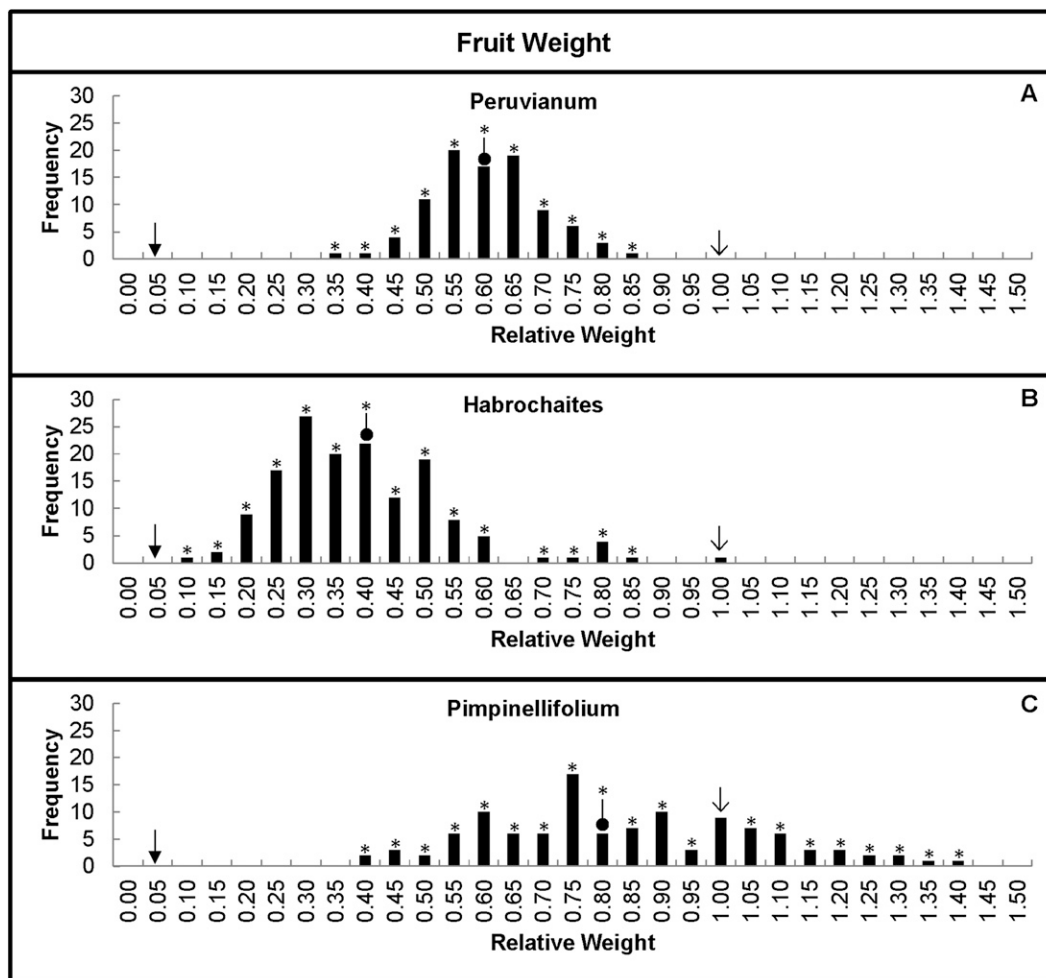


Fig. 4. Fruit weights of (A) *S. peruvianum* LA2172, (B) *S. habrochaites* LA1223, and (C) *S. pimpinellifolium* LA1589-derived populations relative to *S. lycopersicum*. Cultivated parent, wild-type parent, and mean of the population are shown with open, closed, and spherical arrows, respectively. Asterisks indicate bins with real values significantly different from *S. lycopersicum* as determined with Dunnett's method for means comparison ($P \leq 0.05$).

Table 1. Antioxidant trait correlation matrices for Peruvianum, Habrochaites, and Pimpinellifolium populations.^z

		AOX	PHE	VITC	FW
Peruvianum	AOX	1.00	—	—	—
	PHE	0.29	1.00	—	—
	VITC	0.52	0.47	1.00	—
	FW	-0.17	-0.16	-0.29	1.00
Habrochaites	AOX	1.00	—	—	—
	PHE	0.47	1.00	—	—
	VITC	0.44	0.35	1.00	—
	FW	-0.19	-0.18	-0.04	1.00
Pimpinellifolium	AOX	1.00	—	—	—
	PHE	0.60	1.00	—	—
	VITC	0.53	0.51	1.00	—
	FW	-0.19	-0.38	-0.28	1.00

^zAll data are statistically significant at $P \leq 0.05$.

AOC = water-soluble antioxidant; PHE = phenolic; VITC = vitamin C; FW = fresh weight.

than *S. lycopersicum*. For the Habrochaites population, the *S. habrochaites* accession had similar firmness as cultivated tomato (Supplemental Fig. 2B), whereas a total of 36% of the population exceeded the firmness of the *S. lycopersicum* fruit. *S. lycopersicum* fruit were also firmer than *S. pimpinellifolium* fruit (Supplemental Fig. 2C). In addition, 65% of the population had better firmness than the *S. lycopersicum* accession.

External and internal color. EC and IC were determined as a moderate red color for the *S. lycopersicum* accessions, whereas the *S. peruvianum* accession had green fruits. The mean EC and IC for the Peruvianum population were medium red (Supplemental Fig. 3A–B). There was a wide range of variation for both characters ranging from orange to dark red. A total of 57% of the population had darker red EC than cultivated tomato and 36%

of the population exhibited higher IC than *S. lycopersicum*. The *S. habrochaites* accession had green IC and EC; however, none of its progeny had green fruit. Means for both IC and EC for this population were light red (Supplemental Fig. 3C–D). Although 17% of the Habrochaites population had better EC than *S. lycopersicum*, 13% showed better IC. The *S. pimpinellifolium* accession had darker red IC and EC than *S. lycopersicum* (Supplemental Fig. 3E–F). For IC, 6% of the population and for EC 29% of the population had higher values than *S. lycopersicum*.

Correlation of traits. The correlation matrices in Table 1 show that each species exhibited a positive correlation between antioxidant traits. The highest correlation for Peruvianum population antioxidant traits was between WAOX activity and VITC content. However, this association was only moderate ($r = 0.52$). The Habrochaites population showed moderate correlations between WAOX activity and PHE content and between WAOX activity and VITC content. Among the three species, the Pimpinellifolium population had the highest correlations for antioxidant traits. The highest value ($r = 0.60$) was for the association between WAOX activity and PHE content with slightly

Table 2. Agronomic trait correlation matrices for Peruvianum, Habrochaites, and Pimpinellifolium populations.^z

		IC	EC	FIRM	FS
Peruvianum	IC	1.00	—	—	—
	EC	0.75	1.00	—	—
	FIRM	0.23	0.37	1.00	—
	FS	0.38	0.32	0.09	1.00
Habrochaites	IC	1.00	—	—	—
	EC	0.88	1.00	—	—
	FIRM	0.06	0.07	1.00	—
	FS	0.19	0.21	0.02	1.00
Pimpinellifolium	IC	1.00	—	—	—
	EC	0.68	1.00	—	—
	FIRM	0.18	0.25	1.00	—
	FS	-0.13	0.19	0.32	1.00

^zAll data are statistically significant at $P \leq 0.05$.

IC = internal color; EC = external color; FIRM = fruit firmness; FS = fruit shape.

lower values for the correlations between total antioxidant activity and VITC content and between PHE and VITC contents.

There were very few strong correlations between phenotypic traits (Table 2). The highest correlation in all species was found to be between IC and EC. Each species showed strong IC and EC correlations with the Habrochaites population having the highest value ($r = 0.88$) followed by the Peruvianum ($r = 0.75$) and Pimpinellifolium ($r = 0.68$) populations. Moderate correlations were observed between FW and FS in the Peruvianum and Pimpinellifolium populations. Interestingly, these two traits had a negative correlation in the Habrochaites population ($r = -0.31$). Antioxidant traits and FW tended to be negatively correlated.

Discussion

Tomato is an economically and nutritionally important crop and is produced and consumed in large quantities around the world. The main goal of this project was to explore accessions of three wild Solanum species for improvement of antioxidant and agronomic traits in *S. lycopersicum*. For this purpose, three different interspecific populations of *S. peruvianum*, LA2172, *S. habrochaites* LA1223, and *S. pimpinellifolium* LA1589, were analyzed for antioxidant and agronomic traits. Antioxidants are substances that are capable of delaying or inhibiting oxidation processes caused by free radicals. Thus, antioxidant compounds effectively help protect organisms from oxidative stress-mediated damage (Percival, 1998). In humans and other animals, antioxidants are reported to decrease the risk of diseases that are associated with oxidative stress such as cardiovascular diseases, cancers, and neurological disorders (Podsdek, 2007; Yao et al., 2004). Moreover, antioxidants affect plant health. Studies showed that there is a correlation between tomato antioxidants and stress tolerance, especially salinity tolerance (Frery et al., 2010; Mittova et al., 2002, 2004). Therefore, development of tomato cultivars with high WAOX can affect both human and plant health.

The *S. peruvianum*, *S. habrochaites*, and *S. pimpinellifolium* accessions all had

significantly higher antioxidant compound activities/contents than the *S. lycopersicum* lines with only one exception: VITC content in *S. habrochaites* LA1223. This difference between the cultivated and wild species accessions may be explained in two ways. First, domestication and breeding of *S. lycopersicum* involved selection for agronomic traits and may have resulted in the concomitant loss of favorable antioxidant alleles in cultivated tomato. Alternatively, because antioxidants can provide abiotic and biotic stress tolerance, alleles for higher levels of antioxidant compounds may have accumulated in wild species through natural selection and adaptation to the environment. When the wild species were compared among each other, *S. peruvianum* LA2172 had the highest WAOX activity and PHE contents. Despite this, the mean and range of WAOX activities seen in the *S. peruvianum* population were not better than the other species' populations. In fact, the *S. habrochaites* population showed the most range in values and higher proportions of positive transgressive segregation for all three antioxidant traits. These factors are important because they are indicators of the usefulness of each species for cultivated tomato improvement.

The results suggest that among the species accessions tested, *S. habrochaites* LA1223 should be selected as a donor parent for the development of elite lines with high WAOX activity because introduction of alleles from this species gave the best mean improvement in population WAOX activity and the greatest indication that new allele combinations could provide substantial transgressive segregation. Thus, the use of this accession would allow greater incremental improvement of the trait in cultivated tomato. Similarly, *S. habrochaites* LA1223 should be selected for improvement of PHE content in elite lines. Although introduction of alleles from *S. habrochaites* LA1223 did not give a higher mean PHE content than the other wild species, the magnitude of potential improvement as indicated by transgressive segregation was higher. Moreover, the mean VITC content in the Habrochaites population was higher than both parents, which could be explained by heterosis. *S. peruvianum* LA2172 alleles could be used to improve VITC content of

cultivated tomato because the Peruvianum population had the most transgressive segregation and individuals with twice as much VITC as the cultivated tomato line.

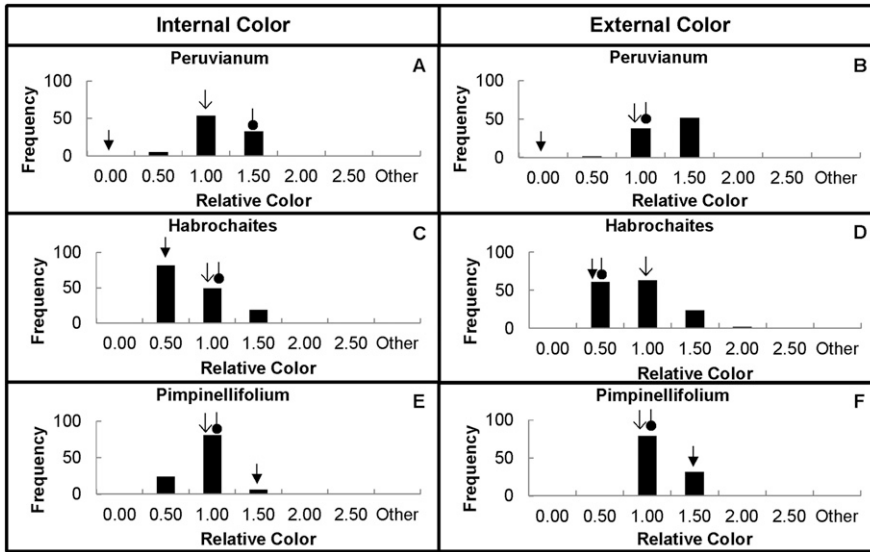
There were moderate positive correlations between WAOX activity and PHE content, PHE content and VITC content, and WAOX activity and VITC content. VITC and PHE are water-soluble antioxidants; therefore, genes responsible for these traits would also be expected to increase WAOX activity. Previous studies also indicated similar positive correlations between PHE content and WAOX activity in tomato and pepper (Frery et al., 2008, 2010; Hanson et al., 2004; Rousseaux et al., 2005; Toor et al., 2006). A positive correlation between VITC content and PHE content was also described in pepper by Frery et al. (2008). Negative correlations were seen between antioxidant traits and FW as observed by Hanson et al. (2004). In general, previous work reported stronger correlations than the ones observed here. These weaker correlations may be the result of greater experimental error or more heterozygosity in the tested populations. This may also explain why the more homozygous population, Pimpinellifolium, had higher antioxidant trait correlations than the other populations. Interestingly, mapping of antioxidant-related quantitative trait loci indicated that *waox6.1*, *vitc6.1*, and *phe6.1* were located on the same part of chromosome 6, whereas *waox12.1*, *vitc12.1*, and *phe12.1* were localized on chromosome 12 (Okmen et al., 2011). Colocalization of the genes controlling these traits may also explain why these antioxidant characters are positively correlated.

Improvement of tomato cultivars for antioxidant characteristics cannot be at the expense of yield and quality traits. For this reason, we also examined these traits in the wild species populations. As expected, the FW of the population progeny was negatively affected by introduction of wild species alleles. Interestingly, the Pimpinellifolium population is the only one that showed transgressive segregation with some individuals having larger fruit than the *S. lycopersicum* parent. IC and EC were most negatively affected in the *S. habrochaites* population.

In summary, our work shows that wild tomato species harbor alleles that could be useful for improvement of antioxidant traits in cultivated tomato. In fact, some of the backcross progeny from the populations used in this study could be used for breeding of these traits. However, as known from more than 50 years of work with tomato wild species, care must be taken that yield and fruit quality traits are not adversely affected by linkage drag. Thus, breeding should be facilitated by use of marker-assisted selection. This is possible because antioxidant traits have been mapped in *S. habrochaites* (Okmen et al., 2011), *S. pimpinellifolium* (Tamer, 2010), and *S. pennellii* (Frery et al., 2010; Rousseaux et al., 2005) with strong indications that such loci locations are conserved across species (Frery et al., 2010; Okmen et al., 2011).

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Supplemental Fig. 3. (A-F).