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Exploring Wild Tomato Species for Morphological Traits, Mineral Elements and Known Disease Resistance Genes

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ABSTRACT

Tomato is one of the worldwide major foods consumed fresh, cooked, or processed. Mineral elements, vitamins, and antioxidant content of tomatoes are of interest because of their nutritional value and beneficial health effects. The present study was performed to evaluate the macro- and micro-elements contents of leaves and fruits of seven wild tomato species, in addition to morphological traits. Wild tomato species had variations for all elements in fruits. Coefficient of variation was calculated for elements as 18.50 to 94.32% for potassium and phosphor, respectively. Most of the wild tomato species had higher content of all mineral elements than cultivated tomato. Resistance genes (*Frl, I-2, I-3, Mi-3, Pto Ty-1, Ty-3* and *Sw-5*) of wild tomato species were screened using molecular markers. LA1971, with six resistant genes, and LA1393 and LA1777, with five resistant genes, were considered the most promising parental candidates for breeding. The results of the analysis of mineral elements of seven wild tomatoes species are useful for future tomato breeding.

Keywords: Mineral elements, Parental selection, Marker assisted selection, Molecular markers.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops worldwide and it belongs to the Solanaceae family (Peralta and Spooner, 2006). The estimated global production is over 163 million tons from around 4.83 million hectares (FAO, 2017). Tomato is one of the major foods consumed fresh, cooked, or processed. It has health beneficial effect due to high content of vitamins A, B, and C, plant carotenoids and sterols such as beta carotene, beta cryptoxanthin, phytosterols, lutein, zeaxanthin, and antioxidant compounds such as lycopene, ascorbic acid, and phenolic acids (Ensminger *et al.,* 1995; Toor *et al.,* 2006).

Consumption of tomatoes, as well as other fresh vegetable and fruits, can prevent chronic diseases, cancer and cardiovascular diseases (Mertz, 1982; Abushita *et al.,* 1997; Dyshlyuk *et al.,* 2020; Etminan *et al.,* 2004; Giovannucci, 1999; Pandey *et al.,* 1995). Vitamins and antioxidant compounds are considered fruit quality traits and, therefore, they are important in tomato breeding. Many phytochemical and QTL mapping studies were performed to determine favourable alleles originating from wild tomato species such as *Solanum pimpinellifolium* (LA1589), *S. habrochaites* (LA1223), and *S.*

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peruvianum (LA2172) to increase in the vitamin and antioxidant content of cultivated tomato (Top *et al.,* 2014). The mineral content is also important because of their nutritional value. For plants, some mineral elements are considered macronutrients (N, P, K, Ca, Mg, S), (Davies and Hobson, 1981; Capel *et al.,* 2017). These are essential for optimum development and growth of tomato due to their presence in many macromolecules such as proteins and enzymes (Sainju *et al.,* 2003). These elements also influence fruit quality, such as flavor and firmness (Dorais *et al.,* 2001; Capel *et al.,* 2017).

Some mineral elements are classified as microelements and are essential for tomato growth also because they can act as a cofactor of enzymes. In addition, microelements are also cofactors of antioxidant enzymes and have scavenging function of free radicals produced during oxidative stress. Thus, micro elements have a beneficial health effect (Leung 1998; Fraga, 2005; Fernández-Ruiz *et al.,* 2011; Sarker *et al.,* 2014; Sarker *et al.,* 2015; Keles *et al.,* 2016). There are different studies aimed to characterize the mineral content of tomato and its wild relatives. It has been reported that there is variation among tomato cultivars in terms of mineral elements content (Suárez *et al.,* 2007; Violeta *et al.,* 2013; Chávez-Servia *et al.,* 2018). Also, QTLs for mineral content originating from *S. pimpinellifolium* were identified (Capel *et al.,* 2017). These studies suggested that wild tomato species have favourable alleles for increase in mineral elements content. In previous research, the genetic potential of three wild tomato species (*S. cheesmaniae, S. habrochaites and S. pimpinellifolium*) was reported for mineral elements content (Fernández-Ruizet *et al.*, 2011). However, there are still more wild tomato species that need to be assessed. The present study aimed to evaluate the mineral elements content of 16 accessions of seven wild tomato species. In addition, some morphological traits and resistance genes of these wild tomato species were characterized.

MATERIALS AND METHODS

Sixteen accessions of seven wild tomato species (two accessions of *S. lycopersicoides* and *S. chilense*, four accessions of *S. habrochaites* and *S. pimpinellifolium*, one accession of *S. sitiens,* two accessions of *S. peruvianum* and one accession of *S. chmielewskii*) were grown in a greenhouse in Antalya Turkey (Figure S1). Accession numbers and origin of the seeds of all wild genotypes are listed in Table 1 and were sown in contained peat and perlite mixture (3:1). Seedlings of plants were grown in a greenhouse using a randomized complete block design (random blocks) with two replications, each consisting of 10 plants. Cultural applications, irrigation, and fertilizer and pesticide applications were done as described by Jones *et al.* (1991). Plants from each replicate were evaluated for 21 morphological traits (quantitative and qualitative traits) according to UPOV (2015) (International Union for the Protection of New Varieties of Plants) (Table 2).

Mineral Elements Contents

Mineral elements contents were evaluated in fruits and leaves. Leaf sampling was carried out by taking the fifth or sixth leaves of the plant from the top. In addition, fruit sampling was made from fruits that reached harvest maturity to represent each plant. For determination of mineral nutrient composition, tomato leaves and fruits bulked from two replications were bulked and rinsed with distilled water after washing with tap water. They were dried in an air-forced oven (Memmert Beschickung-100-800) at 65°C to constant weight (Kacar, 1972). After that, dried leaves and fruits were grinded for further analysis. Dried leaf and fruit samples of 0.5 g each were digested with 10 mL $HNO_3/HClO_4$ (4:1) acid

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Table 1. Accession numbers of wild tomato species.

Accession Number	Name of species	Code	Origin of seeds[a]	Accession number	Name of species	Code	Origin of seeds[a]
LA2408	*S. lycopersicoides*	1	TGRC	LA1974	*S. sitiens*	26	TGRC
LA1964	*S. lycopersicoides*	2	TGRC	LA2656	*S. pimpinellifolium*	30	TGRC
LA4117A	*S. chilense*	12	TGRC	LA0442	*S. pimpinellifolium*	31	TGRC
LA1971	*S. chilense*	13	TGRC	LA1579	*S. pimpinellifolium*	32	TGRC
LA0407	*S. habrochaites*	20	TGRC	LA2093	*S. pimpinellifolium*	33	TGRC
LA1778	*S. habrochaites*	21	TGRC	LA2744	*S. peruvianum*	35	TGRC
LA1393	*S. habrochaites*	22	TGRC	LA0462	*S. peruvianum*	36	TGRC
LA1777	*S. habrochaites*	23	TGRC	LA1318	*S. chmielewskii*	40	TGRC

[a] TGRC= Tomato Genetic Resource Center.

Table 2. Morphological traits, scale and abbreviation of tomato plants.

Morphological traits	Scale	Abbreviation
Plant attitude	Weak, medium, strong	PA
Seedling: Anthocyanin coloration of hypocotyl	Absent, present	SA
Inflorescence type	Uniparous, forked, multiparous or irregular	IT
Length of stem at first inflorescence (cm)	Average of 10 plants	LS
Plant growth habit	Determinate, indeterminate	PG
Plant stem thickness (mm)	Average of 10 plants	ST
Stem hairs	Weak, medium, strong	SH
Flower sepal color	Yellow, orange	FS
Fruit green shoulder (before maturity)	Absent, present	GS
Leaf: length (cm)	Average of 10 plants	LL
Leaf width (cm)	Average of 10 plants	LW
Leaf: intensity of green color	Light, medium, dark green	LC
Leaf: attitude	Semi erect, horizontal, drooping	LA
Leaf type	1,2,3,4 type	LT
Fruit color intensity at maturity	Light green, medium green, dark green, red	FC
Fruit weight (g)	Average of 10 fruits	FW
Predominant fruit shape (After the fruit turns color)	Flattened, oblate, circular, oblong, elliptic, or obovate	PS
Fruit width (mm)	Average of 10 fruits	FWH
Fruit length (mm)	Average of 10 fruits	FLH
Fruit number of seeds	Weak, medium, strong	FNS

mixture on a hot plate (Gestigkeit HD 3-20). The samples were then heated until a clear solution was obtained, and the same procedure was performed several times. The samples were filtered and diluted to 100 mL using distilled water. Total Nitrogen content

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(N) was determined by Kjeldahl digestion according to Bremner (1965); concentrations of potassium (K), Calcium (Ca), Magnesium (Mg), iron (Fe), Zinc (Zn), Manganese (Mn) and Copper (Cu) in the digests were determined using inductively coupled plasma (Perkin Elmer Optima DV7000-ICP OES) (Kacar and Inal, 2008). Phosphorus (P) was measured by a spectrophotometer (UV-VIS SpectrophotometerPG Instruments T60) (Kacar and Kovancı, 1982). Mineral elements contents of fruits and leaves were presented in dry weights (dw) as percentages (%) and mg kg^{-1}, respectively.

Cluster Analysis

Cluster analysis was performed using UPGMA provided by DARwin software. Distance matrix was calculated by using Euclidean coefficient (Perrier and Jacquemoud-Collet 2006).

Resistance Gene Screening

The resistance genes of all accessions of wild tomato species were screened using molecular markers tightly linked to resistance genes and loci. Resistance genes used in the study were Frl for fusarium crown rot disease caused by *Fusarium oxysporum* f. sp. *radicis lycopersici* (Mutlu *et al.,* 2015); *I-2* and *I-3* for race 2 and 3, respectively of *Fusarium oxysporum* f. sp. *lycopersici* (Fusarium wilt) (Staniaszek *et al.,* 2007; Hemming *et al.,* 2004); *Mi* for root-knot nematodes (El Mehrach *et al.,* 2005); *Pto* for bacterial speck *Pseudomonas syringae* pv. *tomato* "Okabe" Y. D. & W (Yang and Francis, 2005); *Ty-1* and *Ty-3* for Tomato Yellow Leaf Curl Virus (TYLCV) (De Castro *et al.,* 2007; Ji *et al.,* 2007) and *Sw-5* for Tomato Spotted Wild Virus (TSWV) (Dianese *et al.,* 2010) (Table 3). Genomic DNA was extracted from fresh leaf tissue using a CTAB method, and disease resistance assays were performed accosting to respective publications (Doyle and Doyle, 1987).

RESULTS

All accessions of seven wild tomato species were evaluated for eight quantitative ((Length of stem at first (LS), Plant stem thickness (ST), Fruit green shoulder (GS), Leaf: length (LL), Leaf width (LW), Fruit weight (FW), Fruit width (FWH), Fruit length (FLH) and 12 qualitative traits (Plant attitude (PA), Seedling: anthocyanin coloration (SA), Inflorescence type (IT), Plant growth habit (PG), Stem hairs (SH), Flower sepal color (FS), Leaf: intensity of green color (LC), Leaf: attitude (LA), Leaf type (LT), Fruit color intensity at maturity (FC), Predominant fruit shape (PS), Fruit number of seeds (FNS)) sentence was rewritten with definition of abbreviation. Coefficient of Variation (CV) ranged from 25.2 (leaf length) to 57.7% (fruit weight). Fruit weight, which is the most important quantitative trait, ranged from 0.2 g (*S.*

Table 3. Disease resistance gene and reference source of primer.

Disease	Resistance gene	References
Fusarium oxysporum f. sp. *radicis lycopersici*	*Frl*	Mutlu *et al.,* 2015
Nematod	*Mi*	El Mehrach *et al.,* 2005
Fusarium oxysporum f. sp. *lycopersici*	*I-2*	Staniaszek *et al.,* 2007
	I-3	Hemming *et al.,* 2004
Tomato yellow leaf curl virus	*Ty-1*	De Castro *et al.,* 2007
	Ty-3	Ji *et al.,* 2007
Tomato spotted wilt virus	*Sw-5*	Dianese *et al.,* 2010
Pseudomonas syringae pv. *tomato*	*Pto*	Yang and Francis, 2005

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lycopersicoides LA2408) to 4.8 g (*S. habrochaites* LA1778) with a mean value of 2.16 g (Table S1). For qualitative morphological traits, CV ranged from 42.37 (predominant fruit shape) to 88.19% (leaf attitude). There was no variation in plant growth habit, flower sepal color, and leaf type (Table 4 and Table S4). Cluster analysis based on all morphological traits generated a dendrogram that contained two clusters (Clusters A and B). Cluster A contained *S. pimpinellifolium* (LA2656, LA0442, and LA2093) *S. peruvianum* (LA2744), *S.sitiens* (LA1974), *S. lycopersicoides* (LA2408) and *S. chilense* (LA1971). Cluster B contained *S. peruvianum* (LA0462), *S. chilense* (LA4117A) and *S. habrochaites* (LA0407, LA1393, and LA1777) accessions (Figure S2).

Table 4. Descriptive statistics of morphological traits.

	Plant attitude	Seedling: Anthocyanin	Inflorescence type	Plant growth habit	Stem hairs	Flower sepal color	Leaf: intensity of green color	Leafattitude	Leaf type	Fruit color intensity at maturity	Predominant fruit shape	Fruit number of seeds	Fruit number of seeds
Average	3.00	1.88	4.00	3.00	3.63	1.00	3.63	1.50	1.00	4.13	3.63	2.00	2.00
Standard deviation	1.73	0.99	1.73	0.00	2.09	0.00	1.83	1.32	0.00	2.23	1.54	1.73	1.73
CV	57.74	52.92	43.30	0.00	57.60	0.00	50.56	88.19	0.00	54.12	42.37	86.60	86.60
Min	1.00	1.00	1.00	3.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Max	5.00	3.00	5.00	3.00	7.00	1.00	5.00	5.00	1.00	7.00	5.00	5.00	5.00

Mineral Elements Contents

The macro-elements (N, P, K, Ca, Mg) and micro-elements (Cu, Fe, Zn, Mn) contents in leaves and fruits of wild tomato species were analyzed separately. The nitrogen content of leaves of all wild tomato accessions and species was higher than cultivated tomato. *S. lycopersicoides*_1 had the highest nitrogen content (5.96%, 2.18 fold higher than cultivated tomato). Phosphorus content of all wild tomato species was lower than cultivated tomato, except for *S.lycopersicoides*_1. The potassium content of all plant material was higher than cultivated tomato, similar to nitrogen content. *S. sitiens*_26 and *S. pimpinellifolium*_30 had the highest potassium content (5.04 and 4.97%, 4.80 and 4.73 times higher than cultivated tomato, respectively). All wild tomato species had higher calcium content than cultivated tomato, except for *S. sitiens*_26 and *S. pimpinellifolium*_30. None of the wild tomato species had higher magnesium content than cultivated tomato. Among the microelements, the iron content of leaves of all plant material was higher than cultivated

tomato, except for *S. lycopersicoides*_2., *S. pimpinellifolium*_30, and *S. chilense*_13 had the highest iron content (347 and 341.6 mg kg^{-1}, 3.07 and 3.03 times higher than cultivated tomato, respectively). *S. lycopersicoides*_1 had the highest zinc content (93.8 mg kg^{-1}, 2.87 fold higher than cultivated tomato). *S. pimpinellifolium*_31 and *S. peruvianum*_36 had the highest manganese content (277.8 and 234.2 mg kg^{-1}, 2.50 and 2.11 fold higher than cultivated tomato. respectively). The copper content of all plant material was lower than cultivated tomato, except for *S. lycopersicoides*_1 and *S. peruvianum*_35 (14.6 and 28 mg kg^{-1}, 1.26 and 2.41 times higher than cultivated tomato, respectively) (Table 5).

Calcium content moderately correlated (ranged from r^2= -0.669 to 0.547) with all mineral content and trace elements, except for iron and copper (negatively correlated to N, P, K, Zn and positively correlated with Mg and Mn) (Table S2). Also, there were moderate correlations between N and P, Ca, Zn and Mn (positive correlation for P and Zn, and negative correlations for Ca and Mn) (Table S2).

Hierarchical clustering was performed based on macro-elements (N, P, K, Ca, Mg) and micro-elements (Cu, Fe, Zn, Mn) content in leaf. There was a high correlation between distance and tree matrix (R^2= 0.93) based on the Mantel test. As a result, the dendrogram contained two clusters (Clusters A and B). *S. lycopersicoides* (LA1964) and *S. lycopersicum* were not clustered. Cluster A contained two subclusters (Subcluster 1A and 2A). Subcluster 1A contained all *S. habrochaites* (LA1777, LA1778, LA1393 and LA0407) accessions and *S. lycopersicoides* (LA2408) accession. Subcluster 2A contained two accessions of *S. chilense* (LA4117A and LA1971) and one accession of *S. sitiens* (LA1974) and *S. chmielewskii* (LA1318).Cluster B contained all *S. pimpinellifolium* accessions, except for

Table 5. Mineral elements composition of wild tomato species and cultivated tomato leaves.

	N	P	K	Ca	Mg	Fe	Zn	Mn	Cu
	% (dw)					mg kg^{-1} (dw)			
*S. lycopersicoides*_1	5.96	0.28	3.33	3.00	0.33	241.20	93.80	125.20	14.60
*S. lycopersicoides*_2	4.42	0.09	1.79	4.40	0.21	47.80	67.20	179.00	4.00
*S. chilense*_12	4.15	0.10	2.60	4.34	0.28	297.20	44.40	108.00	5.60
*S. chilense*_13	3.78	0.09	3.03	4.75	0.26	341.60	68.00	96.20	2.80
*S. habrochaites*_20	4.05	0.09	2.78	5.05	0.13	280.00	32.40	151.20	4.40
*S. habrochaites*_21	4.27	0.11	1.78	4.38	0.18	244.80	24.00	134.40	7.20
*S. habrochaites*_22	4.55	0.11	2.98	4.84	0.18	249.80	22.80	168.40	9.80
*S. habrochaites*_23	3.85	0.14	1.63	5.53	0.18	238.40	36.00	152.20	4.20
*S. sitiens*_26	4.89	0.15	5.04	2.82	0.34	299.20	56.00	52.80	6.20
*S. pimpinellifolium*_30	2.83	0.16	4.97	2.49	0.23	347.00	49.00	181.40	10.60
*S. pimpinellifolium*_31	2.99	0.10	2.30	7.54	0.42	189.80	32.00	277.80	5.60
*S. pimpinellifolium*_32	2.96	0.07	1.69	9.52	0.54	318.80	18.80	215.00	5.20
*S. pimpinellifolium*_33	3.16	0.06	1.90	7.85	0.47	340.40	10.40	143.00	4.40
*S. peruvianum*_35	3.76	0.12	2.55	6.03	0.19	313.60	43.60	195.00	28.00
*S. peruvianum*_36	2.97	0.09	2.92	5.81	0.18	312.60	33.80	234.20	2.00
*S. chmielewskii*_40	3.84	0.12	3.26	5.43	0.14	263.80	36.00	96.00	4.80
Cultivated tomato	2.74	0.25	1.05	2.86	0.57	112.87	32.73	111.07	11.60

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Table 6. Mineral elements composition of wild tomato species and cultivated tomato fruits.

	N	P	K	Ca	Mg	Fe	Zn	Mn	Cu
	% (dw)					mg kg^{-1} (dw)			
*S. lycopersicoides*_1	3.23	0.62	3.79	0.51	0.12	178.50	81.02	26.14	10.98
*S. lycopersicoides*_2	2.48	0.38	2.77	0.38	0.09	96.24	78.20	25.18	7.83
*S. chilense*_12	3.79	0.57	4.03	0.23	0.13	81.04	104.20	24.28	14.61
*S. chilense*_13	4.42	0.68	4.18	0.43	0.18	111.70	100.80	30.15	10.05
*S. habrochaites*_20	3.88	19.80	3.37	0.20	0.10	43.20	11.20	15.20	6.20
*S. habrochaites*_21	3.86	0.57	3.38	0.47	0.17	80.87	65.07	25.50	15.47
*S. habrochaites*_22	3.81	0.43	3.45	0.24	0.13	82.22	100.50	24.45	15.19
*S. habrochaites*_23	4.17	14.14	2.30	0.15	0.07	65.80	5.60	9.40	2.40
*S. sitiens*_26	2.28	0.22	2.43	0.15	0.06	93.16	59.49	13.55	8.49
*S. pimpinellifolium*_30	3.30	28.18	2.94	0.12	0.12	62.60	7.60	16.60	5.40
*S. pimpinellifolium*_31	3.04	14.21	2.50	0.08	0.12	65.00	9.00	12.20	2.40
*S. pimpinellifolium*_32	3.68	11.70	2.45	0.13	0.10	59.40	2.60	16.20	1.40
*S. pimpinellifolium*_33	4.71	19.44	3.70	0.07	0.15	40.00	12.20	16.20	7.20
*S. peruvianum*_35	5.22	24.74	3.18	0.09	0.13	50.60	17.80	15.20	6.00
*S. peruvianum*_36	3.60	18.45	3.48	0.11	0.14	43.20	11.60	17.20	2.60
*S. chmielewskii*_40	5.84	32.54	4.01	0.10	0.13	57.40	14.80	11.60	6.80
Cultivated tomato	1.83	0.27	2.48	0.13	0.09	8.53	17.80	9.60	5.40

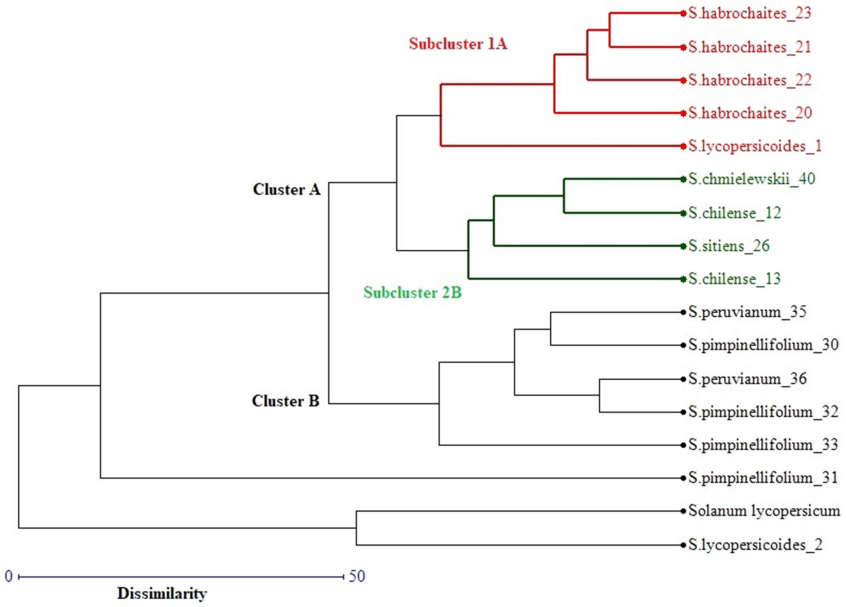

Figure 1. Dendrogram based on mineral and trace elements content of leaves.

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one accession (LA0442) and *S. peruvianum* (LA2744) (Figure 1). Distance between *S. lycopersicoides* (2) and *S. chilense* (23) was highest.

The macroelements (N, P, K, Ca, Mg) and microelements (Cu, Fe, Zn, Mn) contents of the fruits of wild species were determined as indicated in Table 6. Nitrogen content of fruits of all wild tomato accessions and species was higher than cultivated tomato. *S. chmielewskii*_40 and *S. peruvianum*_35 had the highest nitrogen content (5.84 and 5.22%, 3.20 and 2.86 times higher than cultivated tomato, respectively). Phosphorus content of all plant material was higher than cultivated tomato, except for *S. sitiens*_26. *S. chmielewskii*_40 had the highest phosphorus content (32.54%, 119.96 times higher than cultivated tomato). For potassium content, *S.chilense*_13, *S. chilense*_12 and *S. chmielewskii*_40 had the highest value (4.18, 4.03, and 4.01%, 1.69, 1.63 and 1.62 times higher than cultivated tomato, respectively). *S. lycopersicoides*_1 had the highest calcium content (0.51%, 3.88 times higher than cultivated tomato). For magnesium content, *S.chilense*_13 and *S.habrochaites*_21 had the highest value (0.18 and 0.17%, 2.04 and 1.93 times higher than cultivated tomato, respectively).

Among the micro elements, iron content of fruits of all plant material was higher than cultivated tomato. *S. lycopersicoides*_1 and *S. chilense*_13 had the highest iron content (178.50 and 111.70 mg kg^{-1}, 20.93 and 13.09 times higher than cultivated tomato, respectively). For zinc content, *S. chilense*_12, *S. chilense*_13 and *S. habrochaites*_22 had the highest value (104.20, 100.80, and 100.50 mg kg^{-1}, 5.85, 5.66, and 5.65 times higher than cultivate tomato, respectively). *S. chilense*_13 had the highest manganese content (30.15 mg kg^{-1}, 3.14 times higher than cultivated tomato). *S. habrochaites*_21 and *S. habrochaites*_22 had the highest copper content (15.47 and 15.19 mg kg^{-1}, 2.86 and 2.81 times higher than cultivated tomato, respectively). The highest positive correlation was determined between calcium and Fe, Zn, Mn, and Cu, and calcium, which was negatively correlated to P (Table S3).

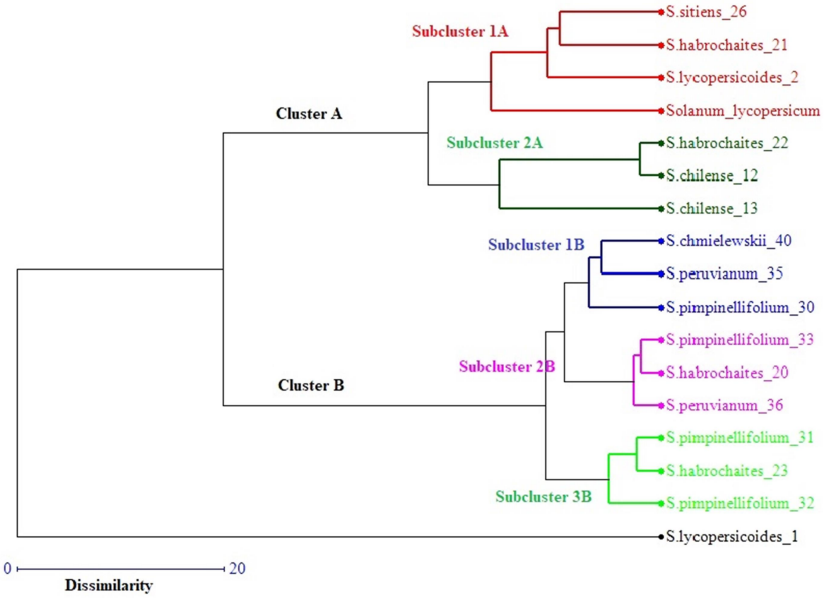

Figure 2. Dendrogram based on mineral and trace elements content of fruits.

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Table 7. Accession number, species and resistance genes.[a]

Accession number	Species	Code	*Mi3*	*SW-5*	*Ty-1*	*I2*	*I3*	*Ty-3*	*Frl*	*Ve*
LA2408	*S. lycopersicoides*	1	R	-	-	-	R	-	-	S
LA1964	*S. lycopersicoides*	2	-	-	-	-	R	-	S	S
LA4117A	*S. chilense*	12	S	R	-	-	H	-	-	S
LA1971	*S. chilense*	13	R	R	R	R	R	R	S	S
LA0407	*S. habrochaites*	20	S	R	S	R	R	-	H	R
LA1778	*S. habrochaites*	21	S	S	S	R	R	-	R	R
LA1393	*S. habrochaites*	22	S	H	S	R	R	S	H	R
LA1777	*S. habrochaites*	23	S	R	S	R	R	S	H	R
LA1974	*S. sitiens*	26	S	-	-	-	-	-	-	R
LA2656	*S. pimpinellifolium*	30	-	S	S	H	H	S	R	S
LA0442	*S. pimpinellifolium*	31	S	S	S	-	H	S	R	S
LA1579	*S. pimpinellifolium*	32	-	S	S	H	H	S	R	S
LA2093	*S. pimpinellifolium*	33	S	S	S	H	H	S	R	S
LA2744	*S. peruvianum*	35	H	R	R	-	H	S	S	S
LA0462	*S. peruvianum*	36	H	R	S	-	R	-	S	S
LA1318	*S. chmielewskii*	40	-	H	S	-	H	S	R	-

[a] R= Resistant, S= Susceptible, H= Heterozygote, -= PCR not obtained.

Hierarchical clustering of wild tomato species based on mineral and trace element content of fruits was performed. The dendrogram contained two clusters (Clusters A and B). Cluster A had two subclusters (1A and 2A). Cluster B contained three subclusters (1B, 2B, and 3B). There was no species-specific clustering pattern. The accessions of wild tomato species were distributed throughout the clusters such as *S. habrochaites* and *S. pimpinellifolium*. As expected, *S. lycopersicoides* was not clustered (Figure 2).

Resistance Gene Screening Using Linked Molecular Markers

Screening of wild tomato species revealed that *S. lycopersicoides* accessions had just *Mi3* and *I3* resistance genes. At least one accession of *S. chilense* had most of the resistance gene, except for *Frl*. Although some of the accessions were heterozygote, *S. habrochaites* had resistance alleles of all resistance genes, except for *Mi3*, *Ty-1* and *Ty-3*. *S. sitiens* (Figure S3). *S. pimpinellifolium* had resistance genes of *I2*, *I3* (both genes were heterozygote) and *Frl*. At least one accession of *S. peruvianum* had resistance genes of *Sw-5*, *Ty-1*, *I-3*, *Frl* and *Mi3* (heterozygote). *S. chmielewskii* had homozygote resistance gene of *Frl* and heterozygote *Sw-5* and *I-3* genes (Table 7).

DISCUSSION

Mineral Element Contents Evaluation

The nutrient concentrations of the leaves and fruits of different tomato species may differ, and the values may be higher in some species and lower in others. The number of

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research on this subject is limited, and there are no limit values for tomatoes fruit components, especially in terms of nutritional concentrations. The nutrient concentrations of the leaves, especially in terms of nitrogen concentration, for *S. lycopersicoides*, *S. chilense*, *S. habrochaites*, *S. sitiens*, *S. peruvianum*_35 and *S. chmielewskii*_40 species were included in the group of high values in the classification by Jones *et al.* (1991). In basal fertilization applications, some species characteristics of tomato plants provided significant increases in nitrogen concentrations in dry matter. Fernandez-Ruiz *et al.* (2011) stated that there is great variability of nutrient concentrations between different tomato species.

Although the P, K, and Mg concentrations of wild-type tomato leaves differ, fertilization applications should be developed specifically for wild tomato species. The deficiency in P concentrations can be caused by lime content and high pH values of the soils (Mengel *et al.*, 2001, Ismail *et al.,* 1996). It has been revealed that deficiencies are observed in some species and species-specific dose trials are required to eliminate the nutrient element deficiencies. The Ca concentrations of all wild tomato leaves were determined at sufficient and high levels, and it is thought that the Ca contents in the soil and the high calcium contents in the irrigation waters make positive contributions (Sainju *et al.,* 2003).

In general, significant nutrient deficiencies were not observed in the microelement concentrations of the tomato leaf samples, and the majority of them were sufficient. Only some tomato species are determined below the limit values, and it will be possible to eliminate these deficiencies with fertilizer applications. Although the sufficiency of microelement concentrations is directly related to soil pH and $CaCO_3$ content, it is possible to provide optimum plant growth by applying fertilizer (Mengel *et al*., 2001; Moraghan and Mascagni, 1991). The nutrient concentrations uptake from the soil by the plants provided sufficient accumulation in the leaves and fruits, and it caused the fruits to have high nutrient content and to increase their nutritive properties. The mean of fruit mineral content of wild tomato species tested in this study were higher in potassium, magnesium, and calcium, but lower in all micro elements (Cu, Fe, Zn, Mn**)** than the three wild tomato species (*S. cheesmaniae, S. habrochaites* and *S. pimpinellifolium*). The differences in mineral content can be due to growing conditions (Guil-Guerrero and Rebolloso-Fuentes, 2009; Hemming *et al.,* 2004; Capel *et al.,* 2017) and due to the use of different accessions of wild tomato species.

The mean of fruit mineral content of wild tomato species tested in this study were higher in potassium, magnesium, and calcium, but lower in all micro elements (Cu, Fe, Zn, Mn**)** than the three wild tomato species (*S. cheesmaniae, S. habrochaites and S. pimpinellifolium*) and cultivated tomato tested by Fernández-Ruiz *et al.* (2011). Also, all wild tomato species analyzed in this study had higher mean content of all micro elements (Cu, Fe, Zn, Mn) than 167 tomato cultivars and 10 F1 tomato hybrids and 11 accessions of cherry-type and medium-sized flattened fruits tested in previous research (Suárez *et al.,* 2007; Violeta *et al.,* 2013; Chávez-Servia *et al.,* 2018).

Resistance Gene Screening Using Linked Molecular Markers

Biotic stress is one of the main problems in tomato production. Natural resistance genes confer resistance to viruses, nematodes, and bacteria and are practical methods in biotic stress management. These resistance genes were identified in wild tomato species and were used in tomato breeding. Interestingly, accessions of the same wild tomato species such as *S. habrochaites, S. chilense* and *S. peruvianum* had different resistance alleles of the genes.

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For genes such as *Mi3, Sw-5,* and *Ty-1*, the present study pointed out the importance of intraspecific variation for resistance genes during parental selections. *S. chilense* **(**LA1971) had six resistance genes out of eight, and the most promising candidate for resistance breeding in tomato after *S. habrochaites* **(**LA1393) and *S. habrochaites* **(**LA1777) had five resistance genes.

CONCLUSIONS

The present study evaluated the mineral and trace element contents of leaves and fruits of the seven wild tomato species to determine the breeding potentials. Most of the wild tomato species had higher content than cultivated tomato for all mineral elements, and wild tomato species had valuable alleles for fruit mineral element breeding. Clustering analysis showed that the mineral content of leaves and fruits did not affect species evolution in tomatoes. In addition, natural resistance genes screening using molecular markers showed that wild tomato species such as *S. chilense* (LA1971) *S. habrochaites* (LA1393) and *S. habrochaites* (LA1777) had most of the resistance genes conferring resistance to various disease factors. The findings of this research are important for integration of wild tomato disease to tomato breeding in terms of mineral content and wild tomato species can be used in tomato breeding in the future.

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بررسی گونه‌های گوجه‌فرنگی وحشی برای ویژگی‌های مورفولوژیکی، عناصر معدنی و ژن‌های مقاوم به بیماری‌های شناخته شده

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چکیده

گوجه فرنگی یکی از خوراکی‌های اصلی در سراسر جهان است که به صورت تازه، پخته یا فرآوری شده مصرف می شود. عناصر معدنی، ویتامین‌ها و محتوای آنتی‌اکسیدانی گوجه‌فرنگی به دلیل ارزش غذایی و اثرات مفید برای سلامتی مورد توجه است.هدف این پژوهش بررسی محتوای عناصرپرمصرف و کم‌مصرف برگ و میوه هفت گونه گوجه فرنگی وحشی و نیز صفات مورفولوژیکی بود. همه عناصر موجود در میوه گونه‌های گوجه‌فرنگی وحشی متفاوت ومتغییربود. ضریب تغییرات برای پتاسیم و فسفر به ترتیب برابر ۱۸/۵۰% تا ۹۴/۳۲% محاسبه شد. اکثر گونه‌های گوجه‌فرنگی وحشی نسبت به گوجه‌فرنگی کشت شده دارای محتوای

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بیشتر عناصر معدنی بودند. ژن های مقاومت (Frl, I-2, I-3, Mi-3, Pto Ty-1, Ty-3 and Sw-5) گونه های گوجه فرنگی وحشی با استفاده از نشانگرهای مولکولی غربال شدند. LA1971، با شش ژن مقاوم، و LA1393 و LA1777، با پنج ژن مقاوم، امیدوار کننده ترین نامزدهای والدین برای اصلاح نژاد در نظر گرفته شدند. نتایج تجزیه و تحلیل عناصر معدنی هفت گونه گوجه فرنگی وحشی برای پرورش گوجه فرنگی در آینده مفید است.

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