Türk Tarım ve Doğa Bilimleri Dergisi 11(2): 583–596, 2024
https://doi.org/10.30910/turkjans.1454880

TÜRK TARIM ve DOĞA BİLİMLERİ DERGİSİ

TURKISH JOURNAL of AGRICULTURAL and NATURAL SCIENCES

www.dergipark.gov.tr/turkjans

Araştırma Makalesi

Screening Organic Acid Contents of Tomato Landraces Collected From Aegean-Mediterranean Region of Anatolia

Seçkin KAYA[1]*, Okan ERKEN[2] Bayram KIZILKAYA[3]

[1]Faculty of Agriculture, Department of Horticulture, Canakkale Onsekiz Mart University, Canakkale, Turkey
[2]Faculty of Agriculture, Department of Farm Structures and Irrigation, Canakkale Onsekiz Mart University, Canakkale, Turkey
[3]Çanakkale Onsekiz Mart University, Faculty of Marine Sciences and Technology, Department of Aquaculture, Çanakkale, Turkey
*Corresponding author: seckinkaya@comu.edu.tr

Geliş Tarihi: 18.10.2024 Düzeltme Geliş Tarihi: 17.04.2024 Kabul Tarihi: 23.04.2024

ABSTRACT

Tomato landraces can serve as valuable sources for breeding new cultivars aimed at enhancing fruit quality in terms of organic acids. Nineteen tomato landraces spread along the Mediterranean coasts of Anatolia were evaluated to determine their basic internal quality parameters and organic acid composition. The parameters assessed included fruit weight, diameter, length, pH, titrable acidity (TA %), soluble solid content (SSC%), SSC/TA ratio, and organic acids, such as oxalic (OA), tartaric (TarA), malic (MA), malonic (MalA), lactic (LA), acetic (AA), citric (CA), and ascorbic acids (AscA). Significant diversity was observed among the different landraces concerning these traits. Citric acid was found to be the most abundant organic acid within the landraces. Notably, the highest and lowest values for CA were recorded in Ege 8 (105.73 mg g-1) and TR62707 (31.10 mg g^{-1}), respectively, making them promising sources for future breeding programs. Ascorbic acid (AscA) exhibited the lowest content among all the organic acids, ranging from 0.06 to 0.12 mg.g^{-1} (equivalent to 64-116 µg.g^{-1}). Moreover, the landrace TR49646 displayed the highest malic acid content (8.23 mg g-1), making it a potential source for obtaining high malic acid content. Conversely, Ege 6 showed the lowest malic acid content (5.11 mg g^{-1}). For health purposes, the landrace TR63233 was identified as having the lowest oxalic acid content. Multidimensional scale analysis further confirmed the potential candidates identified by the ANOVA and one-way ANOM tests. The results revealed a considerable diversity among the evaluated landraces, and the identified traits could be instrumental in selecting and breeding new cultivars with improved characteristics.

Key words: C*itric acid, diversity, organic acids, malic acid, oxalic acid, tomatoes*

Anadolu'nun Ege-Akdeniz Kıyılarindan Toplanan Yerel Domates Çeşitlerinin Organik Asit İçeriklerinin Belirlenmesi

ÖZ

Yerel domates çeşitleri, organik asitler açısından meyve kalitesini arttırmayı amaçlayan yeni çeşitlerin geliştirilmesi için değerli kaynaklar olarak hizmet edebilir. Bu amaçla, Anadolu'nun Akdeniz kıyılarına yayılan 19 yerel domates çeşidi, temel meyve kalite parametreleri ve organik asit içeriklerinin belirlenmesi amacıyla taranmıştır. Çalışma kapsamında yerel domates çeşitlerinde meyve ağırlığı, meyve çapı, meyve boyu, pH, titre edilebilir toplam asitlik (%TETA), suda çözünebilir kuru madde miktarı (%SÇKM), SÇKM/TETA oranı ve oksalik (OA), tartarik (TarA), malik aist (MA), malonik (MalA), laktik (LA), asetik (AA), sitrik (CA) ve askorbik asitler (AscA) içerikleri belirlenmiştir. Elde edilen verilere göre bütün özellikler göz önüne alındığında yerel çeşitler arasında önemli farklılıklar gözlenmiştir. Yerel domates çeşitlerinde beklendiği gibi en fazla bulunan organik asitin, sitrik asit olduğu belirlenmiştir. Dikkat çekici bir şekilde, CA için en yüksek ve en düşük değerler sırasıyla Ege 8 (105,73 mg/g) ve TR62707'de (31,10 mg/g) olarak kaydedilmiş olup, bu da onları gelecekteki ıslah programları için umut verici kaynaklar haline getirmiştir. Askorbik asit (AscA), 0,06 ila 0,12 mg.g-1 (64-116 µg.g-1'e eşdeğer) arasında değişen miktarlar ile tüm organik asitler arasında en az belirlenen asit olmuştur. Ayrıca, TR49646 kod numaralı yerel çeşit en yüksek malik asit (8,23 mg/g) içeren çeşit olarak belirlenmiş ve bu sonuç bu çeşidi yüksek malik asit içeriği elde etmek için ileriki yıllarda kullanılabilecek potansiyel bir genetik kaynak olarak tanımlanmıştır. Bunun tersine, Ege 6 en düşük malik asit içeriğini (5.11 mg/g) göstermiştir. İnsan sağlığı açısından, TR63233 kod numaralı yerel çeşit en düşük oksalik asit içeriğine sahip çeşit olarak belirlenmiştir. Çok boyutlu ölçek analizi, ANOVA ve tek yönlü ANOM testleri tarafından belirlenen potansiyel adayları doğrulamıştır. Sonuçlar, değerlendirilen yerel çeşitler arasında önemli bir çeşitlilik olduğunu ortaya koymuş ve tanımlanan özelliklerin, gelişmiş özelliklere sahip yeni çeşitlerin ıslahında gelecek yıllarda potansiyel olarak kullanılabilir olduğunu ortaya koymuştur.

Anahtar kelimeler: *Sitrik asit, çeşitlilik, organik asit, malik asit, okzalik asit, domates*

INTRODUCTION

Tomatoes are one of the most produced crops with a worldwide production of 186.8 million tons in 2020 (FAO 2021). It is a multipurpose vegetable for both fresh consumption and in the processed food industry, whose production have been increasing each year (Henareh et al. 2015). Due to its economic importance, tomatoes are one of the well-studied vegetable crops scientifically. It has been a good model for applied plant research because of many advantages such as ease of culture under a wide range of environments, short life cycle, self-fertility, homozygosis. These reasons have led tomatoes bred to increase yield and improve quality characteristics and disease resistance. Generally the common goal of tomato breeding is to increase yield per unit area (Foolad 2007). The preference for improved hybrids by farmers with income concerns, however, has restricted landrace production. On the other hand, several landraces are cultivated in Europe and other countries such as Turkey and Iran because of consumers' current interests (Casals et al. 2011*;* Henareh et al. 2015). The entrance history of tomatoes into Anatolia was documented by Bayraktar (1953) and Oraman (1968) who stated that, tomatoes first entered Anatolia from Adana Province in the Mediterranean region in 1770s and spread to other Anatolian regions in the following years. Tan (2010), further stated that there are approximately 80 different tomato accessions stored in gene banks in Turkey whose characteristics are largely undocumented. In addition, today it is possible to find dozens of different tomato landraces in Turkey cultivated by small farmers. However, the cultivation of these old tomato varieties is spread over a large geographic area and their phenotypic and genotypic traits are also undocumented (Karagöz 2003*;* Henareh et al, 2015)*.* Landraces are mostly composed of homozygous genotypes and have significant genetic diversity. Therefore, they show wide genetic variation in both quantitative and qualitative characteristics (Negri et al. 2009*;* Cebolla-Cornejo et al, 2013*;* Reddy et al, 2013). The genetic profiles of landraces are also quite different from those of modern cultivars (Sacco et al. 2015). Landraces have been widely used in crop improvement and breeding programs to uncover valuable traits. Detecting and observing such traits provides guidance to researchers with possible future breeding programs (Tembe et al. 2018). One of the traits is tomato fruit quality, where consumers commonly complain about its taste and flavor (Causse et al. 2003*;* Tandon et al, 2003). Ruiz et al. (2006)*,* reported that tomato taste and flavor have declined because of breeding and that tomatoes have been produced to increase yield, fruit size, firmness, and processing performance and not for organoleptic fruit quality. However, we know that there are many studies conducted on improving the organoleptic quality of tomatoes (Baldwin et al. 1998; Bucheli et al. 1999; Agong et al. 2001; Ruiz et al. 2006; Acosta-Quezada et al. 2015; Henareh et al. 2015). The main components that assess tomato fruit quality are chemical properties such as dry matter, soluble solids, organic acids and volatile components (Thybo et al. 2008). It would not be wrong to say that the organoleptic quality of tomatoes is a combination of flavor, aroma and texture (Causse et al. 2002). According to Marconi et al. (2007) and Thorne and Effiuvwevwere (1998), organic acids have an effect on tomato fruit flavor as well as cultivar, maturity, processing and storage conditions. Tomato fruit contains 5.0-7.0% of dry matter, which includes sugar, organic acids, minerals and vitamins, where organic acids and sugar form the predominance of the total dry matter (Salles et al. 2003*;* Malundo et al. 1995). Organic acids and reducing sugar are also important components that give sweet and sour flavors to tomatoes. Their amount and concentrations can affect the flavor (Davies and Hobson 1981*;* Thorne and Efiuvwevwere 1988) and their levels depend on maturity, growing conditions and cultivars (Baldwin et al. 1991). In addition, they are part of biological routes (Suarez et al. 2008*)* such as the Krebs cycle (Pereira et al. 2013).

Based on the above considerations, the objective of this study was to determine the diversity of the Mediterranean Aegean tomato landraces of Anatolia in terms of organic acid content. Information on this diversity is significant for uncovering germplasm for future tomato breeding and improvement programs.

MATERIAL AND METHODS

Genetic material, description of research site and cultivation conditions

A total of nineteen tomato landraces, collected from different areas in the Aegean-Mediterranean sides of Anatolia, were used as genetic material in the study (Table 1; Figure 1). The tomato landraces were collected and documented in 2012 (Kaya 2012). Tomato landraces named as "Ege" were collected by the researcher in 2009, where the landraces are still cultivated by farmers in the Aegean region of Anotolia. The other samples were collected from the gene bank of the Republic of Turkey Ministry of Agriculture and Forestry, Aegean Agricultural Research Institute located in Izmir, Turkey. The landrace seeds were produced a year before the trial. The total number of seed samples to be included in the trial was determined according to preliminary studies, considering the workforce, status of the trial areas, financial opportunities and healthy follow-up of the trials. A replicated field trial was conducted in the research fields of the Faculty of Agriculture at Çanakkale Onsekiz Mart University, Dardanos, Çanakkale, Turkey. The research site is located in the southwestern part of Anatolia and in the northwestern part of Thrace, at the southern end of the Sea of Marmara. The study area has a transition climate across the Mediterranean and Black Sea. Plants were grown under open field conditions. The soil in which the plants were grown was clay loam structure with 7.21pH, 0.65 mS/cm, 11.20% lime and 1.23% organic matter. The research was planned according to a randomized block design with 3 repetitions and 40 plants in each repetition.

Table 1. Tomato landraces used in the research and their origin information.

Landrace code in gene bank	City	Origin Province
Ege 1	İzmir	Bergama
Ege 3	Manisa	Salihli
Ege 4-	İzmir	Kemalpaşa
Ege 5	İzmir	Kemalpaşa
Ege 6	İzmir	Kemalpaşa
Ege 7	İzmir	Kemalpaşa
Ege 8	İzmir	Kemalpaşa
Ege 9	İzmir	Kemalpaşa
TR49646	İzmir	Kiraz
TR61658	Aydin	Çine
TR61785	Muğla	Fethiye
TR62573	Balikesir	Dursunbey
TR62613	Balikesir	Savaştepe
TR62707	Manisa	Gölmarmara
TR63233	İzmir	Bergama
TR66062	Bursa	İznik
TR69155	Antalya	Korkuteli
TR72500	Adana	Feke
TR72508	Mersin	Uzuncaburç

Figure 1. Tomato landraces used in the research and their origins colored on the map.

Standard cultural practices were used and tomato fruits were harvested at the red maturity stage in 2017 growing season. Tomato fruits were harvested and transferred to the laboratory immediately. A total of 20 fully colored tomato fruits were measured for weight, diameter and length. The fruit weights were determined using the Sartorius precision balance, and the fruit diameter and length were measured using digital caliper. Tomato samples were cut and shaken with a Waring blender to homogenize the tomato samples. Titrable acidity (TA %) was determined from the pulp obtained according to the method defined by Anonymous (1968). pH was determined using an Inolab pH meter, and SSC was determined using Hanna 96801 digital refractometer.

Extraction of Organic Acids

In this study, eight organic acids were investigated and determined from fresh tomato pulp samples. The extraction method of organic acids was performed according to Augustin et al. (1981) with minor modifications. Initially, 10 g of fresh tomato pulp was treated with 50mL of 6% HPO_3 using a magnetic stirrer. The mixture was then filtered with Whatman No. 40 filter paper; the volume was adjusted to 10 ml and passed through a 0.45 µm filter. The extracts were taken into HPLC vials and prepared ready for injection.

HPLC analysis of organic acids

HPLC (Shimadzu, Japan) was used for qualitative and quantitative analysis of organic acids (Arnetoli et al. 2005). SIL- 20AC Auto sampler, LC-20AD pump, SPD-M20A Prominence DAD detector (190-800 nm), CBM-20A system controller, CTO-20AC column oven and LC solution (version: 1.23 sp1) were used in the HPLC system. Chromatographic separation was performed using an Inertsil ODS-III C18 column (4.6x250 ID, 5 µm particle size). The mobile phase was performed with 125 mM KH_2PO_4 adjusted to pH 2.5 with o-phosphoric acid. The column oven was 40^0C and the flow rate of mobile phase was1.4 mL min^{-1}.The wavelength to detect oxalic, tartaric, malic, lactic, acetic and citric acids was 210 nm and wavelength for ascorbic acid was 254 nm. The detection wavelengths were 210 nm for oxalic, tartaric, malic, lactic, acetic, citric, and 254 nm for ascorbic acid (Figure 2). The retention times of organic acids were determined with a mixed solution of all acids for simultaneous determination by preparing a single standard solution at 25 µg.mL^{-1} concentrations. The system was then calibrated with a mixed solution of all organic acids at different concentrations. All procedures were repeated in triplicate.

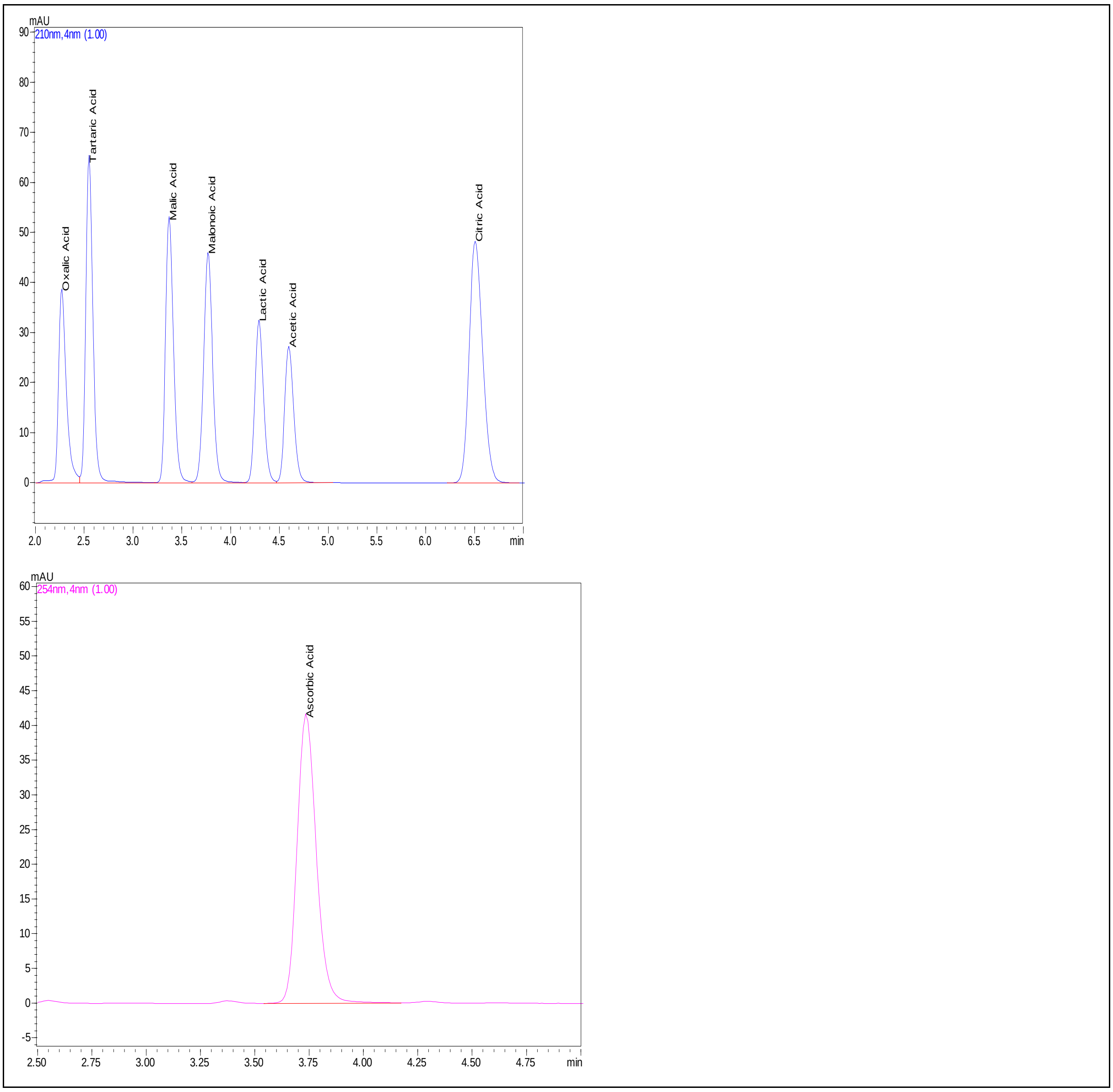

Figure 2. Chromatography Spectra of organic acids (210 nm) and ascorbic acid (254nm).

Statistics

Data were processed by analysis of variance (ANOVA), and Duncan's Multiple Comparison Test was used to distinguish between groups for significant differences in each trait. The SPSS (ver. 16.0) statistical analysis program was used for statistical calculations. After the differences were determined, one-way ANOM graphical tests were performed for the traits of CA, MA and OA which have been found to be statistically significant in ANOVA (Mendeş and Yiğit 2013; Mendeş and Yiğit, 2018). Multidimensional scaling analysis was performed the mean values of each trait for 19 landraces defined by Yiğit and Mendeş (2016).

RESULTS

Fruit size, titrable acidity and soluble solid contents

Significant differences were determined (*$p \leq 0.05$)* among landraces for FW, FD, FL, pH, TA%, SSC% and TA/SSC traits (Table 2). Fruit weights ranged from 150.00 g to 17.33 g when sorted from heavy to light. FD ranged between 85.41 mm to 22.56 mm and FL ranged between 57.47 mm and 24.48 mm among landraces. All traits regarding fruit size were determined statistically significant ($p \leq 0.05$). In addition, the pH of the pulps derived from tomato landraces wase measured between 4.623 and 4.173, which was determined to be statistically significant ($p \leq 0.05$). Important differences were found within the landraces in terms of TA. The highest TA was determined as 0.627% from the landrace labeled TR63233, and the lowest was measured as 0.210 from the landrace TR72508. Similar to the other traits, SSC showed great variation among the landraces and were found to be statistically significant (*$p \leq 0.05$*). SSC of the landraces varied between 7.07% and 4.77%. These wide variations determined for TA and SSC also reflect the SSC: TA ratios.

Organic acids

Oxalic (OA), Tartaric (TarA), Malic (MA), Malanoic (MalA), Lactic (LA), Acetic (AA), Citric (CA) and Ascorbic acid (AscA) contents of 19 tomato landraces are given in Table 3. The wide variation among landraces was determined in terms of organic acids. The OA, MA and CA contents of the landraces were determined statistically significant according to ANOVA tests (*$p \leq 0.05$*). Differences in the TarA, MalA, LA, AA, and AscA content of the landraces were not found to be statistically significant. As expected, citric acid was the predominant organic acid among the organic acids analyzed. The highest CA content was obtained from the landrace Ege 8 at 105.73 mg g^{-1}, and the lowest was obtained from the landrace TR66062 at 20.25 mg g^{-1}. This result shows that there is a 5-fold difference between the least acidic tomato and the most acidic tomato. Similar differences were also determined in the MA contents of the landraces. The lowest MA was determined from the landrace TR63233 as 4.88 mg g^{-1} and the highest MA was determined from the landrace TR49646 as 8.23 mg g^{-1}. OA contents were also found to be statistically significant, but the landrace TR63233 is notable for its low OA content.

Table 2. Mean values of the ANOVA for differences among landraces at least significant difference (*Duncan: p≤0.05)* for fruit quality traits measured in 19 tomato landraces**.**

Genotype Code	Fruit Weight (g)		Fruit Diameter (mm)		Fruit Length (mm)		pH		TA%		SSC%		SSC/TA	
Ege 1	128.87	***c***	63.22	***d***	55.21	***b***	4.433	***e***	.457	***f***	5.03	***gh***	11.02	***gh***
Ege 3	100.20	***g***	22.56	***p***	25.48	***j***	4.357	***g***	.490	***d***	5.07	***gh***	10.34	***hi***
Ege 4	24.33	***q***	85.41	***a***	53.56	***c***	4.603	***ab***	.400	***i***	5.73	***e***	14.34	***d***
Ege 5	148.87	***a***	75.21	***b***	47.43	***e***	4.373	***fg***	.487	***de***	5.07	***gh***	10.41	***hi***
Ege 6	73.56	***l***	55.93	***i***	41.43	***g***	4.580	***bc***	.330	***k***	6.43	***bc***	19.55	***b***
Ege 7	121.30	***d***	58.25	***h***	51.62	***d***	4.623	***a***	.433	***g***	5.20	***g***	12.01	***f***
Ege 8	37.33	***o***	34.56	***n***	31.92	***h***	4.403	***ef***	.557	***b***	6.03	***d***	10.84	***h***
Ege 9	135.70	***b***	62.27	***de***	57.47	***a***	4.483	***d***	.370	***j***	4.93	***hi***	13.34	***de***
TR49646	150.00	***a***	70.60	***c***	50.61	***d***	4.417	***e***	.530	***c***	6.30	***c***	11.89	***fg***
TR61658	94.67	***i***	59.36	***gh***	50.08	***d***	4.550	***c***	.337	***k***	5.47	***f***	16.24	***c***
TR61785	84.67	***j***	52.24	***j***	42.01	***g***	4.347	***g***	.530	***c***	7.07	***a***	13.34	***de***
TR62573	28.67	***p***	36.35	***m***	31.39	***h***	4.357	***g***	.470	***ef***	5.70	***e***	12.13	***f***
TR62613	97.00	***h***	55.30	***i***	47.97	***e***	4.480	***d***	.460	***f***	6.27	***c***	13.63	***de***
TR62707	17.33	***r***	30.91	***o***	27.60	***i***	4.473	***d***	.427	***gh***	5.47	***f***	12.81	***ef***
TR63233	50.33	***m***	43.57	***k***	31.34	***h***	4.173	***h***	.627	***a***	6.07	***d***	9.68	***i***
TR66062	104.63	***f***	61.08	***ef***	45.62	***f***	4.343	***g***	.403	***i***	5.57	***ef***	13.80	***de***
TR69155	82.00	***k***	51.00	***j***	45.18	***f***	4.473	***d***	.410	***hi***	5.50	***f***	13.41	***de***
TR72500	40.77	***n***	40.58	***l***	31.92	***h***	4.583	***bc***	.330	***k***	6.50	***b***	19.75	***b***
TR72508	112.63	***e***	59.84	***fg***	53.37	***c***	4.573	***bc***	.210	***l***	4.77	***i***	22.73	***a***
Significance		**		**		**		**		**		**		**

**Values followed by different letters in a column were significantly different (*p≤0.01)* using Duncan's multiple range test.

Table 3. Mean values of the ANOVA for differences among landraces at least significant difference (*Duncan: p≤0.05*) organic acids ($mg.g^{-1}$) traits and total organic acid per 100 g portion (g/100g) measured in 19 tomato landraces.

Genotype Code	OA ($mg.g^{-1}$)		TarA ($mg.g^{-1}$)	MA ($mg.g^{-1}$)		MalA ($mg.g^{-1}$)	LA ($mg.g^{-1}$)	AA ($mg.g^{-1}$)	CA ($mg.g^{-1}$)		AscA ($mg.g^{-1}$)	Total organic acid per 100 g portion (g/100g)
Ege 1	0,65	**c-e**	2,40	7,08	**b-e**	0,09	8,16	7,94	41,15	**e-g**	0,06	6,75
Ege 3	0,63	**c-e**	3,49	7,00	**b-e**	0,08	14,79	19,14	74,03	**bc**	0,07	11,92
Ege 4	0,73	**a-e**	1,47	6,52	**c-f**	0,07	35,84	13,20	44,66	**ef**	0,07	10,26
Ege 5	0,76	**a-d**	0,21	6,44	**c-g**	0,07	29,81	9,73	64,91	**cd**	0,07	11,20
Ege 6	0,94	**a**	2,79	5,11	**hi**	0,04	13,41	4,88	78,12	**b**	0,08	10,54
Ege 7	0,85	**a-c**	0,15	6,74	**c-f**	0,05	12,86	3,95	49,00	**e**	0,08	7,37
Ege 8	0,78	**a-d**	3,82	8,03	**ab**	0,06	15,02	9,23	105,73	**a**	0,07	14,28
Ege 9	0,81	**a-d**	3,21	6,55	**c-f**	0,05	37,39	4,99	61,13	**cd**	0,10	11,42
TR49646	0,69	**c-e**	2,37	8,23	**a**	0,08	18,21	5,94	64,67	**cd**	0,09	10,03
TR69155	0,60	**de**	1,41	6,65	**c-f**	0,06	24,10	7,85	35,54	**fg**	0,06	7,63
TR62573	0,78	**a-d**	2,73	6,25	**d-h**	0,09	32,68	9,35	38,81	**e-g**	0,10	9,08
TR62613	0,68	**c-e**	2,13	7,45	**a-c**	0,09	29,37	4,17	43,38	**e-g**	0,12	8,74
TR62707	0,68	**c-e**	4,49	5,34	**g-i**	0,08	31,32	6,44	31,10	**gh**	0,10	7,96
TR61658	0,71	**b-e**	1,31	6,09	**e-h**	0,07	40,44	3,63	38,68	**e-g**	0,09	9,10
TR61785	0,67	**c-e**	1,58	5,29	**g-i**	0,06	31,51	4,98	42,85	**e-g**	0,09	8,70
TR63233	0,54	**e**	1,66	4,88	**i**	0,04	18,59	5,91	47,26	**ef**	0,10	7,90
TR66062	0,93	**ab**	3,36	6,00	**e-i**	0,05	59,12	12,28	20,25	**h**	0,09	10,21
TR72500	0,94	**a**	3,80	5,62	**f-i**	0,01	34,70	9,60	40,79	**e-g**	0,12	9,56
TR72508	0,91	**ab**	3,50	7,34	**a-d**	0,05	51,75	7,98	47,26	**ef**	0,10	11,89
Average	**0.75**		**2.42**	**6.45**		**0.06**	**28.37**	**7.96**	**51.02**		**0.09**	**-**
DTL	**0.12**		**1.22**	**0.95**		**0.02**	**13.56**	**3.86**	**19.75**		**0.02**	**-**
%ADTL	**16.02**		**50.45**	**14.70**		**31.48**	**47.78**	**48.45**	**38.72**		**18.86**	
Significance	********		***n.s***	********		***n.s***	***n.s***	***n.s***	********		***n.s***	**-**

**Values followed by different letters in a column were significantly different (p ≤0.01) using Duncan's multiple range test. ns: not significant; OA: oxalic acid; TarA: tartaric acid; MA: malic acid; MalA: Malonoic acid; LA: lactic acid; AA: Acetic acid; CA: citric acid; AscA: ascorbic acid. All statistical calculations are based on $mg.L^{-1}$ unit over the extraction solution according to HPLC results). DTL: Value of Deviation between Tomato Landraces; %ADTL: Percent Deviation of Average with respect to DTL

DISCUSSION

In general, consumers do not like the taste of modern tomato cultivars and claim that many heirloom varieties have better taste and aroma quality (Tieman et al. 2012). This is because the nutritional levels of fruits and vegetables decrease as a result of intensive breeding studies (Klee and Tieman 2013). From viewpoint, it is necessary to investigate the sources of variation for the traits of interest (Acosta-Quezada et al. 2015) because today's modern varieties can be developed in terms of taste and aroma (Ruiz et al. 2005). On the other hand, the improvement and commercialization of landraces may empower small-scale farmers and enable them to generate more income (Agong et al. 2001). For this purpose, many studies have been conducted to understand and document the composition of tomato fruit and its variation (Mata et al. 2000; Schauer et al. 2005; Ruiz et al. 2006; Marconi et al. 2007; Suarez et al. 2008; Pareira et al. 2013; Acosta-Quezada et al. 2015). The study discussed below is the first to examine the diversity of organic acids in of local tomato landraces spread to the Aegean-Mediterranean side of Anatolia.

The mean values of fruit weight (g), length (mm) and fruit diameter (mm) are provided in Table 2. According to these results, great variation was determined among 19 landraces. For instance, a more than 8-fold difference was found between the weights of the smallest and largest tomato fruits. If we describe the fruit size as a combination of three traits such as weight, diameter and length, it is easy to say that the differences between fruit size also prove the genetic diversity among the landraces. The same landraces were previously studied by Kaya (2012), and great variability was determined among the landraces. The size of the fruit may be related to whether the plant is genetically inclined to bloom or not (Cavicchi and Silvetti 1976; Grandillo et al. 1999). However, this situation can also be affected by growing conditions such as fertilizing. Because the growing conditions in these studies were kept as constant as possible, it would not be wrong to say that the reason for the diversity in fruit sizes is genetic factors.

Titrable acidity (TA) screened in tomato fruits among landraces showed wide diversity. The landrace that contains the highest TA (TR63233) may be suggested as a good source for breeding programs in the future to increase TA in fruits. In contrast, the landrace that gave the lowest TA (TR72508) may also be a candidate to improve fruit quality, especially for tree tomatoes (Boyes and Strübi 1997). However, TA ratio alone is not a sufficient indicator for future studies to improve taste. Researchers generally focused on SSC, TA and pH and attempted to increase these traits (Hewitt and Garvey 1987; Triano and St Calair 1995). Moreover scientist have attempted to explain the relationships between the traits such as acidity, SSC, sugars, to determine the components of tomato taste (Baldwin et al. 1991; Malunda et al. 1999; Agong et al. 2001; Marconi et al. 2007; Acosta- Klee & Tiaman 2013; Quezada et al. 2015). The tomato taste is a complex of several components, and it can be said that, sweetness is directly affected by SSC and reducing sugars, while sourness is affected by the amount of soluble solid content, pH and TA (Stevens et al. 1977, 1979; Baldwin et al. 1998). Galiana-Balaguer et al. (2006), cited that it is important to know which genes control the traits while planning a breeding study in tomatoes. Genes that control traits such as TA and SSC are polygenic which creates some difficulties in breeding programs (Saliba Colombani et al. 2001; Fulton et al. 2002). Like TA, SSC is another important trait for taste and quality of tomatoes. The range of variation of SSC in 19 tomato landraces was between 4.77% and 7.07%. Our study is in agreement with previous research (Mata et al. 2000; Agong et al. 2001; Galiana-Balaguer et al. 2006; Kaya 2012).

The organic acid content of the tomato landraces was analyzed by ANOVA (Table 3), but it is not sufficient to determine which landraces are different from others. Therefore, One-way ANOM tests were carried out with data that were found statistically significant with the ANOVA test determines the upper and lower decision lines for the landraces. Our results regarding the predominant organic acid being citric acid are in agreement with previous studies, as expected (Thorne and Effiuvwevwere 1998; Marconi et al. 2007; Acosta-Quezada et al. 2015). Furthermore, the wide variation among landraces was determined in terms of citric acid. The One way ANOM graph of the citric acid content of the landraces is shown in Figure 3. The results show that significant differences and wide variation were detected among landraces ($p \leq 0.05$) in terms of CA. The highest CA content was determined in the Ege 8 (105.73 mg g^{-1}), Ege 6 (78.12 mg g^{-1}), Ege 3 (74.03 mg g^{-1}), TR49646 (64.67 mg g^{-1}) and Ege 5 (64.91 mg g^{-1}) landraces. These 5 landraces were determined over the range of the upper decision line according to the one way normal ANOM test. In contrast three, landraces (TR66062, TR62707 and TR69155) had the lowest CA contents. According to the results obtained, the five highest landraces that gave the highest citric acid concentration may be suggested as a source for a breeding program. Organic acid concentrations in tomato vary according to maturity and the cultivar (Baldwin et al. 1991). Tomato fruits had been harvested at the red maturity phase for each accession in our trials, so it may be claimed that the variation between the accessions depends largely on genetic factors. In addition, Choi et al. (2014) attributed phenotypic differences in tomatoes largely to genetic factors. These variations in the composition profile of citric acid can be used for the introgression of favorable traits from landraces into the genetic background of cultivated species. It is claimed that, tomato taste intensity is perceived to be higher when the amount of sugar and organic acids are higher (Stevens et al. 1977; Bucheli et al. 1999; Galiana-Balaguer et al. 2006). However, citric acid concentration is not the only indicator of a breeding program. The taste and flavor of tomato is the sum of sugars, acids, and many other volatile chemicals. These chemicals could affect consumer preferences (Klee and Tieman 2013). For instance, the ratio of malic to citric acid should be lower than 0.5. At higher levels, the taste turns sour because malic acid has been reported to be approximately aproximately14% sourer than citric acid (Petro-Turza 1987; Yılmaz 2001). Therefore, dozens of factors affecting the taste and aroma of tomatoes should be considered and breeding programs should be carefully planned.

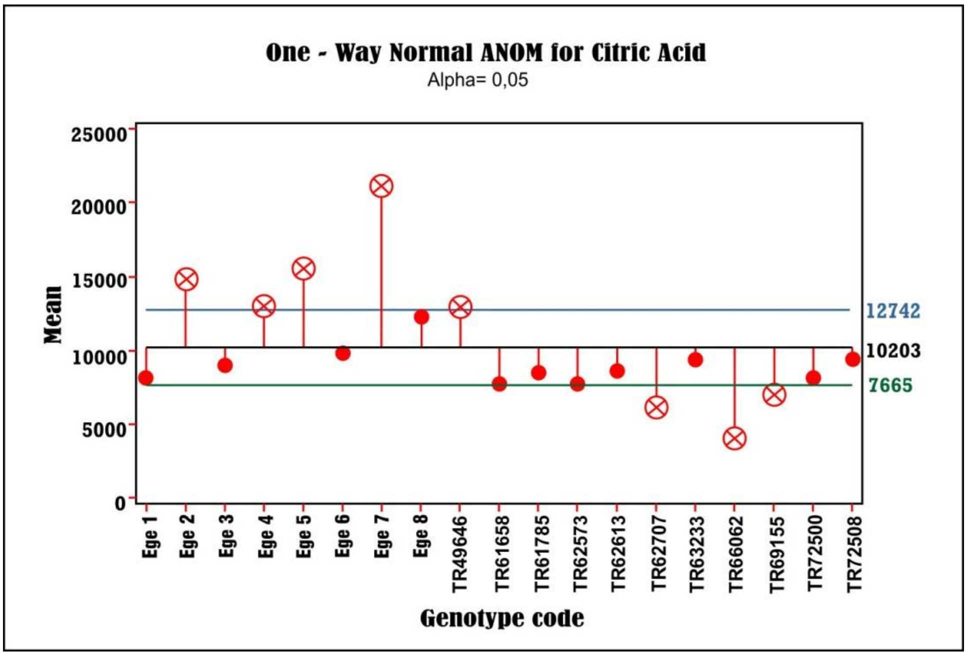

Figure 3. One way ANOM test graph for citric acid concentrations of the landraces

A One way ANOM graph of the malic acid content of the landraces is shown in Figure 4. The results show that significant differences and wide variation were detected among landraces (*p≤0.05)* in terms of malic acid. The highest malic acid contents were determined in TR49646 (8.23 mg g^{-1}) and Ege 8 (8.03 mg g^{-1}) landraces. In addition, these two landraces were over range according to one way normal ANOM test, in which the upper limit had been determined as 1514.5 mg L^{-1} in extraction solution. The results show that, these two landraces have significantly higher malic acid contents than the other landraces. The average malic acid content of the landraces was 6.45 mg g^{-1}. On the other hand, 3 landraces, TR63233, Ege 6 and TR61785, gave the lowest malic acid contents of 4.88, 5.11, 5.29 mg g^{-1}, respectively. These landraces are below the lower decision line of 1067.10 mg L^{-1} calculated by the one way ANOM test (*p≤0.05)* in the extraction solution. The MA content of the landraces is in agreement with other studies (Suarez et al. 2008; Mata et al. 2000; Galiana-Balaguer et al. 2006; Breksa III et al. 2015). The varieties labeled TR49646 and Ege 8 can be recommended as sources of high malic acid content. One of the interesting results of the ANOM test is that, Ege 8 and TR49646 are above the upper decision line for both CA and MA. Fulton et al. (2002) reported positive correlations between malic acid and citric acid, providing yet another proof of the relationship between CA and MA.

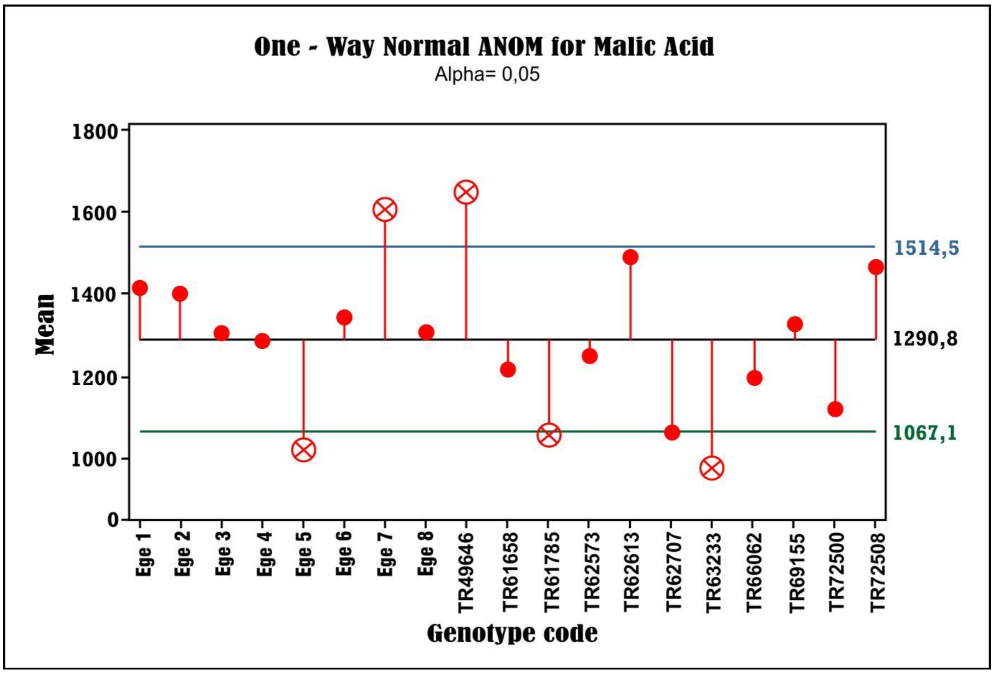

Figure 4. One way ANOM test graph for malic acid concentrations of the accessions.

A One way ANOM graph of the oxalic acid content of the landraces is shown in Figure 5. Results show that we have found narrow diversity among landraces in terms of oxalic acid content, but a statistical significance was (*p≤0.05*) detected. The highest oxalic acid content was determined in Ege 6 (188.96 mg L^{-1}). This result, however, was not over the upper decision line,therefore, it can be said that this landrace is consistent with the other landraces except for landrace TR63233. The landrace TR63233 was determined under the lower decision line, which means that only this landrace was different from the others. Suarez et al. (2008) determined the OA contents in 5 tomato cultivars between 25 and 37.5 mg L^{-1}, but Mata et al. (2000) determined a wide range for OA content of tomato cultivars and accessions between 270 mg kg^{-1} and 2580 mg kg^{-1} (with another unit expression as 0.27-2.58 mg g^{-1}). Our results, which are between 0.54 and 0.94 mg g^{-1} are in agreement with those studies. The importance of OA in tomato composition should not be overlooked because it is important from a human nutrition point of view. Generally, the amount of OA in food should be low. OA diminishes the bioavailability of calcium in the alimentary canal (Guil et al. 1996). For instance, 1 g of calcium precipitates with 2.5 g of oxalic acid. Therefore, the bioavailability of calcium is affected by oxalic acid. If the relationship between calcium and oxalic acid is greater than 2.25, it is considered as an insufficient source of calcium for food (Mitjavila 1990). Therefore from this viewpoint, TR63233 can be recommended as a source of low OA content.

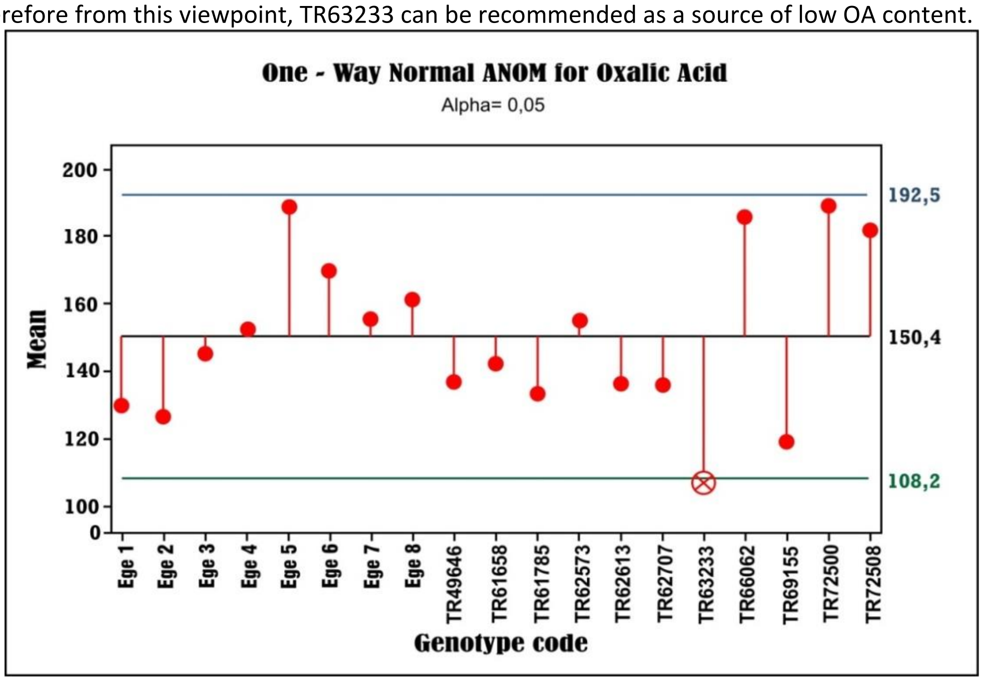

Figure 5. One way ANOM test graph for oxalic acid concentrations of the accessions.

The multidimensional scaling analysis showed a wide diversity among landraces, and distinguished those that were almost similar or dissimilar. It can be said that the MDS analysis is a reliable tool in explaining and assigning similarities among landraces (Figure 6). 11 landraces, circled as A, showed similarities or narrow diversity among each other and 5 landraces, circled as B, also showed similarities or narrow diversity among each other. However, the landraces grouped together as A and B showed a wide variation. One of the interesting results is that landraces named Ege 8, TR72508 and TR66062 differed from all other landraces and showed great diversity from the other 16 landraces. When data are examined carefully, it can be seen that TR66062 has the lowest CA content, Ege 8 has the second highest TA and highest CA content, and TR72500 has the third highest OA and second lowest SSC content. Thus, the places and group of landraces on the MDS analysis diagram can be explained. Overall, the results indicate that wide diversity among landraces exists for the traits studied. The data explained above may provide ideas and prospects for the selection or use of these landraces as germplasm sources in the future.

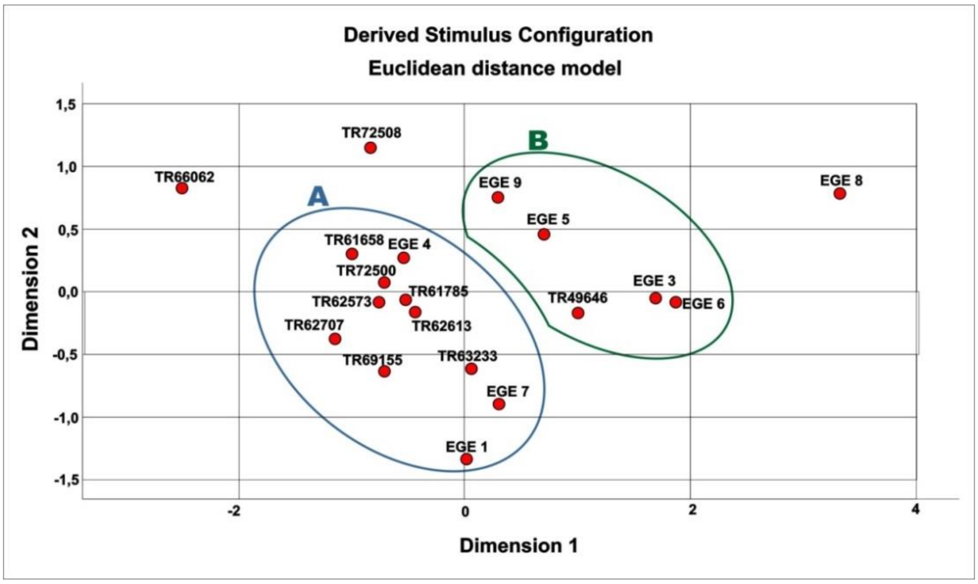

Figure 6. A multidimensional scaling analysis (MDS) diagram shows the relationships among the 15 traits for 19 landraces. Results are based on the 19 landraces obtained for which data were available for all traits studied (RSQ=0.99580).

CONCLUSIONS

Many researchers have stated that the systematic evaluation and characterization of local tomato gene sources is essential for current and future agricultural studies and genetic progression. Determining the traits of landraces and heirloom varieties will increase the knowledge of researchers on issues such as how much genetic variability is available and how it will be easier to conduct breeding studies in wider geographical areas. Internal quality and the taste of tomato is a difficult issue and should be explained. The growing interest of people in food quality is a driving force for the improvement of more nutritious and tastier foods. However, the genetic richness found in landraces and heirloom varieties can be used to develop new cultivars. In this study, organic acids in tomato landraces in the Mediterranean coastline of Anatolia were determined. According to the results obtained, wide diversity was detected among the 19 landraces studied. In addition, high CA content in landraces Ege 8, Ege 6, Ege 3, TR49646 and Ege 5 was found to be promising. Moreover, TR49646 and Ege 8 were determined as MA sources for future research. TR63233 was also determined as the tomato landrace with the lowest OA content.

Acknowledgements: This study was supported by Canakkale Onsekiz Mart University, The Scientific Research Coordination Unit, project grand number FHD-216-911. Authors would like to thank to Dr. Tutku AK for English editing and to Dr. Soner YIGIT for statistical analysis.

Conflict of Interests: The authors declare that no competing interests exist.

Authors' Contributions: Seçkin Kaya designed the experiments and obtained funding as project manager. Seçkin Kaya, Okan Erken and Bayram Kızılkaya analyzed the samples at laboratory together. Also Seçkin Kaya wrote the manuscript. All authors read and approved the final manuscript.

AUTHORS' ORCID NUMBERS

Seçkin KAYA https://orcid.org/0000-0003-2157-7215

Okan ERKEN https://orcid.org/0000-0001-5177-7432

Bayram KIZILKAYA https://orcid.org/0000-0002-3916-3734

REFERENCES

Acosta-Quezada, P.G., Raigon, M.D., Riofrio-Cuenca, T., Garcia-Martinez, M.D., Plazas, M., Burneo, J.I., Figueroa, J.G., Vilanova, S., Prohens, J., 2015. Diversity for chemical composition in a collection of different varietaltypes of tree tomato (*Solanum betaceum*), an Andean exotic fruit. Food Chem 169: 327-335.

Agong, S.G., Shittenhelm, S., Friedt, W., 2001. Genotypic variation of Kenyan tomato (*Lycopersicon esculentum* L.) germplasm. The J of Food Tech in Africa 6; 13–17.

Anonymous, 1968. International Federation of Fruit Juice Producers No: 3.

Arnetoli, M., Montegrossi, G., Bucciant, A., Gonnelli, C., 2008. Determination of organic acids in plants of *Silene paradoxa* l. by HPLC. J Agric Food Chem 56: 789–795.

Augustin, J., Beck, C., Marousek, G.I., 1981. Quantitative determination of ascorbic acid in potatoes and potato products by high performance liquid chromatography. J of Food Sci 46: 312-316.

Baldwin, E.A., Nisperos-Carriedo, M.O., Baker, R., Scott, J.W., 1991. Quantitative analysis of flavour parameters in six parameters in six Florida tomato cultivars (*Lycopersicon esculentum* Mill.). J Agric Food Chem 39: 1135–1140.

Baldwin, E.A., Scott, J.W., Einstein, M.A., Malundo, T.M.M., Carr, B.T., Shewfelt, R.L., and Tandon, K.S., 1998. Relationship between sensory and instrumental analysis for tomato flavor. J Am Soc Horti Sci 123: 906–915.

Bayraktar, K., 1953. Comparative studies on the characteristics and technological values of native and American tomato varieties cultivated in vegetable fields. Ankara University, Faculty of Agriculture 42, Ankara. Work document.

Bayraktar, K., 1966. Vegetable Growing. Vol I. Ege University, İzmir (in Turkish).

Boyes, S., Strübi, P., 1997. Organic acid and sugar composition of three New Zealand grown tamarillo varieties (*Solanum betaceum* (Cav.)). NZ J Crop and Hort. Sci. 25: 79–83.

Breksa, A., Robertson, L., Labate, J., King, B., King, D., 2015. Physicochemical and morphological analysis of ten tomato varieties identifies quality traits more readily manipulated through breeding and traditional selection methods. J Food Comp and Analysis. 42: 16-25.

Bucheli, P., Voirol, E., Torre, R.R., Lopez, J., Rytz, A., Tanksley, S.D., Petiard, V., de la Torre, R., 1999. Definition of non-volatile markers for flavor of tomato (*Lycopersicon esculentum* Mill.) as tools in selection and breeding. J Agric Food Chem 47: 659–664.

Casals, J., Pascual, L., Canizares, J., Cebolla-Cornejo, J., Casanas, F., Nuez, F., 2011. The risks of success in quality vegetable markets: Possible genetic erosion in Marmande tomatoes (*Solanum lycopersicum* L.) and consumer dissatisfaction. Sci Hort 130: 78–84.

Causse, M., Buret, M., Robini, K., Verschave, P., 2003. Inheritance of nutritional and sensory quality traits in fresh market tomato and relation to consumer preferences. J Food Sci 68: 2342–2350.

Causse, M., Saliba-Colombani, V., Lecomte, L., Duffé, P., Rousselle, P., Buret, M., 2002. QTL analysis of fruit quality in fresh market tomato: A few chromosome regions control the variation of sensory and instrumental traits. J Exp Bot 53: 377, 2089-2098.

Cavicchi, S., Silvetti, E.,1976. Yield in tomato. I. Multiple regression between yield and yield components. Gen Agri 30: 293-313.

Cebolla-Cornejo, J., Rosello, S., Nuez, F., 2013. Phenotypic and genetic diversity of Spanish tomato landraces. Sci Hort 162: 150–164.

Choi, S.H., Kim, D.S., Kozukue, N., Kim, H.J., Nishitani, Y., Mizuno, M., Levin, C.E., Friedman, M., 2014. Protein, free amino acid, phenolic, b-carotene, and lycopene content, and antioxidative and cancer cell inhibitory effects of 12 green-house-grown commercial cherry tomato varieties. J Food Comp Analysis 34 (2): 115–127.

Davies, J.N., Hobson, G.E., 1981. The constituents of tomato fruit the influence of environment, nutrition, and genotype. Crit. Rev. Food Sci. Technol. 15: 205–280.

FAO, 2021. Food and agriculture organization of the UN statistics division website. Available: http://www.fao.org/faostat/en/#data/QC/visualize. 20 Dec.2021.

Foolad, M.R., 2007. Genome mapping and molecular breeding of tomato. Int J of Plant Gen. 52.

Fulton, T.M., Bucheli, P., Voirol, E., Lopez, J., Petiard & Tanksley, S.D., 2002. Quantitative trait loci (QTL) affecting sugars, organic acids and other biochemical properties possibly contributing to flavor, identified in four advanced backcross populations of tomato. Euphytica 127: 163–177.

Galiana-Balaguer, L., Rosello, S., Nuez, F., 2006. Characterization and selection of balanced sources of variability for breeding tomato (Lycopersicon) internal quality. Gen Res and Crop Evol 53: 907–923.

Grandillo, S., Zamir, D., Tanskley, S.D., 1999. Genetic improvement of processing tomatoes: A 20 years perspective. Euphytica 110: 85–97.

Henareh, M., Dursun, A., Mandoulakani, B.A., 2015. Genetic diversity in tomato landraces collected from Turkey and Iran revealed by morphological characters. Acta Sci Pol Hort Cultus 14(2): 87–96.

Karagöz, A., 2003. Plant genetic resources conservation in Turkey. Acta Horti 598: 17-25.

Kaya, S., 2012. Researches on determining local table tomato populations available for organic farming and usage opportunities to improve organic cultivars. Doctorate Thesis. Ege University, Turkey.

Klee, H.J., Tieman, D.M., 2013. Genetic challenges of flavor improvement in tomato. Trends in genetics 29: 4.

Malundo, T.M.M., Shewfelt, R.L., Scott, J.W., 1995. Flavor quality of fresh tomato (*Lycopersicon esculentum* Mill.) as affected by sugar and acid levels. Postharvest Biol Technol 6:103–10.

Marconi, O., Floridi, S., Montanari, L., 2007. Organic acids profile in tomato juice by HPLC with UV detection. J of Food Quality 30: 253–266.

Mata, M.C.S., Hurtado, M.C., Rosell, S., Ripolles, S.R., Galiana-Balaguer, L., Isasa, M.E.T., Vifials, F.N., 2000. Breeding for flavour of fresh market tomato: sources for increasing acid content. Acta Physiol Plant 22: 250.

Mendeş, M., Yiğit, S., 2013. Comparison of ANOVA-F and ANOM tests with regard to type I error rate and test power. J of Statistical Comp Simul 83(11): 2093-2104.

Mendeş, M., Yiğit, S., 2018. An alternative approach for multiple comparison problems when there are a large number of groups: ANOM technique. J Animal & Plant Sci. 28: (4).

Mitjavila, S., 1990. Sustancias naturales nocivas en los alimentos. In: Derache J (ed) Toxicologı´a y seguridad de los alimentos. Omega, Barcelona

Mucha, A.P., Almeida, C.M.R., Bordalo, A.A., Vasconcelos, M.T.S.D., 2005. Exudation of organic acids by a marsh plant and implications on trace metal availability in the rhizosphere of estuarine sediments. Est Coast Shelf Sci 65:191–198.

Negri, V., Maxted, N., Veteläinen, M., 2009. European landrace conservation: an introduction. In: Veteläinen, M., Negri, V., Maxted, N. (Eds.), European Landraces:On-farm Conservation Management and Use, Biodiversity Technical Bulletin 15.Bioversity International, Macaresse, pp. 1–22.

Oraman, M.N., 1968. Vegetable science. (in Turkish). Ankara University.

Pereira, C., Barrosa, L., Carvalho, A.M., Isabel, C., Ferreira, F.R., 2013. Use of UFLC-PDA for the analysis of organic acids in thirty five species of food and medicinal plants. Food Anal Methods 6:1337–1344

Petro-Turza. M., 1987. Flavor of tomato and tomato products. Food Rev Int 2 (3): 309-351.

Reddy, B.R., Reddy. M.P., Begum, H., Sunil, N., 2013. Genetic diversity studies in tomato (*Solanum lycopersicum* L.). J Agri Vet Sci 4: 53–55.

Ruiz, J.J., Alonso, A., Garcia-Martinez, S., Valero, M., Basco, P., Ruiz-Bevia, F., 2005. Quantitive analyses of flavour volatiles detects differences among closely related traditional cultivars of tomato. J of Sci Food Agr 85: 54–60.

Ruiz, J.J., Valero, M., García-Martínez, S., Serrano, M., Moral, R., 2006. Effect of recent genetic improvement on some analytical parameters of tomato fruit quality.Comm Soil Sci and Plant Anal 37:15-20, 2647-2658.

Sacco, A., Ruggieri, V., Parisi, M., Festa, G., Rigano, M.M., Picarella, M.E., Mazzucato, A., Barone, A., 2015. Exploring a tomato landraces collection for fruit-related traits by the aid of a high-throughput genomic platform. PLoS ONE 10(9).

Saliba Colombani, V., Causse, M., Langlois, D., Philouze, J., Buret, M., 2001. Genetic analysis of organoleptic quality in fresh market tomato. 1. Mapping QTLs for physical and chemical traits. Theor Appl Gen 102: 259–272.

Salles, C., Nicklaus, S., Septier, C., 2003. Determination and gustatory properties of taste-active compounds in tomato juice. Food Chem 81: 395–402.

Schauer, N., Zamir, D., Fernie, A.R., 2005. Metabolic profiling of leaves and fruit of wild species tomato: A survey of the *Solanum lycopersicum* complex. J Exp Botany 56: 410, 297-307.

Stevens, M.A., Kader, A.A., Albright-Holton, M., Algazi, M., 1977. Genotypic Variation for flavour and composition in fresh market tomatoes. J Am Soc Hort Sci 102: 680–689.

Suarez, M.H., Rodriguez, E.R., Romero, C.D., 2008. Analysis of organic acid content in cultivars of tomato harvested in Tenerife. Eur Food Res Technol 226: 423.

Tan, A., 2010. The status of plant genetic resources for food and agriculture in Turkey. Regarding the conservation and sustainable use of plant resources for food and agriculture, the second country report on Turkey (in Turkish).

Tandon, K.S., Baldwin, E.A., Scott, J.W., Shewfelt, R.L., 2003. Linking sensory descriptors to volatile and nonvolatile components of fresh tomato flavor. J. of Food Sci 68: 2366–2371.

Tembe, K.O., Chemining'wa, G., Ambuko, J., Owino, W., 2018. Evaluation of African tomato landraces (*Solanum lycopersicum*) based on morphological and horticultural traits. Agri and Nat Res 52: 536-542.

Thorne, S.N, Efiuvwevwere, B.J.O., 1988. Changes in organic acids in chilled tomato fruit (*Lycopersicon esculentum* Mill.). J Sci Food Agric 44: 309–319.

Thybo, A.K., Edelenbos, M., Christensen, L.P., Sørensen, J.N., Thorup-Kristensen, 2006. Effect of organic growing systems on sensory quality and chemical composition of tomatoes. LWT-Food Sci Tech 39:835–843.

Tieman, D.M., Bliss, P., McIntyre, L.M., Blandon-Ubeda, A., Bies, D., Odabaşı, A.Z., Rodriguez, G.R., van der Knaap, E., Taylor, M.G., Goulet, C., Mageroy, M.H., et. al, 2012. The chemical interactions underlying tomato flavor preferences. Curr. Biol. 22, 1–5 Elsevier Ltd. Working paper.

Yiğit, S., Mendeş, M., 2016. Usage of multidimensional scaling technique for evaluating performances of multivariate normality tests. British J App Sci & Technol. 16: 1–6

Yılmaz, E., 2001. The chemistry of fresh tomato flavor. Turk J Agric Forestry 25: 149-155.