Article

Biochemical Characterisation and Genetic Structure Provide Insight into the Diversity of the Mediterranean Tomato Ancient Varieties ‘San Marzano’ and ‘Re Fiascone’: New Resources for Breeding

Pasquale Tripodi * , Rosa Pepe, Gianluca Francese, Macellaro Rosaria, Vincenzo Onofaro Sanajà, Carlo Di Cesare, Giovanna Festa, Antonietta D’Alessandro and Giuseppe Mennella

CREA Research Centre for Vegetable and Ornamental Crops, 84098 Pontecagnano, Italy; rosa.pepe@crea.gov.it (R.P.); gianluca.francese@crea.gov.it (G.F.); rosaria.macellaro061@gmail.com (M.R.); vincenzo.onofarosanaja@crea.gov.it (V.O.S.); carlo.dicesare@crea.gov.it (C.D.C.); giovanna.festa@crea.gov.it (G.F.); antonietta.dalessandro@crea.gov.it (A.D.); giuseppe.mennella@crea.gov.it (G.M.)

* Correspondence: pasquale.tripodi@crea.gov.it; Tel.: +39-089-386217

Abstract: Tomato (Solanum lycopersicum L.) is one of the most important vegetable crops highly appreciated for the nutritional properties and content of beneficial compounds for human health. After its domestication, this crop has been spread throughout the world and found its secondary centre of diversification in the Mediterranean basin, where numerous landraces have been developed. Among these, ‘San Marzano’ is a famous and remunerative PDO variety for processing and fresh market consumption. ‘Re Fiascone’ is an ancient cultivar for which ongoing efforts aim to promote its diffusion and commercialisation. Both find their origin in the Campania region in Southern Italy where in the past decades, several accessions have been selected and handed down by farmers. This study reports a comprehensive assessment for morphological and biochemical traits of twenty-one accessions of the two landraces. Fruit morphology and content of sugars and flavonoids were the most discriminating parameters among cultivars. Among sugars and organic acids, fructose and citric acid were the most represented compounds, respectively. We found level of trans-lycopene and β-carotene up to 112.82 and 16.29 µg g⁻¹ of fresh product, respectively, while ascorbic acid levels reached values up to 22.48 mg 100 g⁻¹ of fresh weight. Molecular analysis has been performed using a double approach for microsatellite genotyping based on capillary electrophoresis and high-resolution melting. Results highlighted a separation of the accessions according to ‘Re Fiascone’ and ‘San Marzano’ identifying both unique and admixed accessions between the two groups. The study approach aims at the recovery and valorisation of local genetic resources, but also at the identification of traits of interest to transfer in breeding programs.

Keywords: tomato; landraces; bioactive compounds; lycopene; β-carotene; flavonoids; microsatellites; high resolution melting

1. Introduction

Tomato is a major vegetable crop part of the large Solanaceae family, which includes over 3000 species native to both the Old (eggplant, in China and India) and the New World (tomato, pepper and potato, in Central and South America) [1]. During the XVI century, after the discovery of America through the travels of Spanish conquistadors, the crop found a secondary Centre for diversification in the Mediterranean area of Europe, where selection activities led to the development of a wide array of landraces [2]. Within this region, Italy is a leading country ranking at 6th and 10th in the World for production and area of cultivation, respectively; being furthermore the first European country for both parameters [3].
In the Southern part of Italy, the Campania region represents an important basin for tomato production with a long tradition of selection of local varieties developed according to farmer preferences and adaptation to local edaphic and climatic conditions. Among these, ‘Pomodoro S. Marzano dell’Agro Sarnese-Nocerino’ received the PDO—Protected Designations of Origin (area of cultivation Avellino, Napoli and Salerno in the Campania region) under European Union law namely [4]. This long-shaped landrace is among the most appreciated commodity for its taste, flavour, and aptitude for industrial processing. The unicity of the cultivar is given by a combination of factors, including the fertile soil of volcanic origin and the skills of producers in the production area [4,5]. ‘San Marzano’ was a leading product for the canning industry for tomato peeled sauce, being marketed on the national territory, and exported to numerous countries of Europe and the Americas since the beginning of the twentieth century [6]. Although different accessions have been maintained and handed down over the years by farmers, the cultivation of these tomatoes has significantly reduced due to emerging of viral diseases but also the establishment of high-yielding hybrids better fitting the needs of intensive farming (e.g., adaptable to mechanical harvesting) [7,8].

Another ancient variety historically known as ‘Tomato Re Umberto’ has been established as a tribute to the Italian King Umberto I of Savoia when he visited Naples for the first time in 1878 [9]. Efforts of associations and regional projects, put back in cultivation this landrace, providing it the label ‘Re Fiascone’ [10]. Its area of development and diffusion as well as the properties of tomato for sauce make this tomato considered as a progenitor of ‘San Marzano’.

These landraces are not represented by a pure homozygous line, but by a set of differentiated biotypes developed in the different microenvironments of Campania, following both natural and artificial selection, aimed above all at the production of fruits suitable for peeling. The different ecotypes of the ‘San Marzano’ group are characterised by types of red berry tomatoes and cylindrical, fewer seeds and suitable for the transformation of the product [11], whereas the accessions of the ‘Re Fiascone’ group display fruits with shape mostly rounded or squared and consistent pulp [4]. Such diversity represents a source of useful traits underlying nutritional and organoleptic quality, lacking instead in many improved and commercialised cultivars [12], being therefore a reservoir of potential alleles for breeding for quality. In addition, the direct link with the territory and the intrinsic cultural value make these ancient varieties suitable to be promoted for their direct use, thus enhancing local economies. In recent years a rise has been observed in the consumption of these two landraces for both fresh and processed purposes [4], thus indicating a renewed interest in consumers and farmers. In an agricultural scenario that must cope with climate changes and demographic trends expected in the next decades, a primary task is the promotion of healthy diets from sustainable food systems [13,14]. It is, therefore, necessary to rediscover and explore the potentiality of these local varieties through valorisation programs to boost their production and commercialisation.

To provide a more comprehensive knowledge of the potentialities of ‘San Marzano’ and ‘Re Fiascone’ varieties, we characterised 21 accessions recovered from main farming areas in Campania. The analysis included morphological and chemical traits as well as the biochemical composition and levels of vitamin C, trans-lycopene, β-carotene, flavonoids, sugars and organic acids. These compounds are relevant for human health given the beneficial properties that prevent degenerative disease, representing furthermore, the main precursors of organoleptic quality [15,16].

In addition, we investigated the genetic diversity by implementing capillary electrophoresis and high-resolution melting for genotyping with microsatellites. To our knowledge, this is furthermore the first attempt to assess the ancient ‘Re Fiascone’ and compare it with the most famous ‘San Marzano’.

The approach presented in this study provides an opportunity to identify novel sources of traits from existing biodiversity and promote locally adapted germplasm for breeding for quality and sustainability in the next decades.
2. Materials and Methods

2.1. Plant Material

Plant material consisted of 21 landraces retrieved from farms located in main districts of Campania Region including the Agro Sarnese-Nocerino, the Piana Del Sele, and the Napoli areas (Figure 1). The accessions studied represent the variability occurring within ‘San Marzano’ and ‘Re Fiascone’ based on the area of cultivation and the farms from where the genotypes were retrieved. Plants were grown under plastic tunnels at the experimental farm of the Research Centre for Vegetable and Ornamental Crops located in Pontecagnano (Salerno Province) (40°39’ N 14°53’ E; 28 m above sea level) during spring–summer 2018. Seeds were sown in March and seedlings transplanted in early May in single rows, adopting distances of 100 cm between the rows and 50 cm along the rows. Plants were grown in three replicates and a completely randomised design using six plants/accession for each replicate. Cultivation was managed according to standard agronomic practices; plants were irrigated throughout the entire cultivation period using a drip irrigation system and according to crop evapotranspiration. The cultivation technique included stakes as support and galvanised wires.

![Map of Campania Region](image1)

**Figure 1.** Details of collecting and cultivation site for the ‘San Marzano’ and ‘Re Fiascone’ landraces. (A) The Campania region with three major districts: Napoli (I), Agro Sarnese-Nocerino (II) and Piana Del Sele (III). (B) Details of cultivation at the experimental farm of the Research Centre for Vegetable and Ornamental Crops located in Pontecagnano (SA).

2.2. Fruit Traits and Chemical Assessment

Sampling has been done from each plant, harvesting full ripened fruits from the third truss. In total, a bulk of 30 representative fruits were considered for each accession/replicate. Morphological traits were scored as following: (i) average fruit weight (FW) (g); (ii) fruit...
length (FL) and width (FD) scored by a ruler and fruit shape index (FS) as ratio FL/FD; (iii) thickness of pericarp (TC) measured by a calliper. After being washed and dried, fruits were homogenised using a Waring blender (2-L capacity; Model HGB140, Waring Commercial, Stamford, CT, USA) for 60 sec. Soluble solids content (SSC) was measured using a digital refractometer (Refracto 30PX, Mettler-Toledo, Novate Milanese, Italy), and the results were expressed as °Brix on 100 g of fresh weight (FW). The pH and the titratable acidity (TA) were determined using a pH-Matic 23 titroprocessor equipped with a pH electrode with a temperature sensor (model 5011T) (Crison Instruments, Barcelona, Spain). Titratable acidity was expressed as g citric acid/L juice.

2.3. Biochemical Evaluation

Biochemical measurements were carried out on well-ripe fruits harvested in July-August from the three replicates. Six fruits from each replicate were pooled to achieve representative aliquots (each of 10 g) of fruit sample which was immediately frozen in liquid nitrogen and stored at −80 °C until the analyses. Each biological replicate was analysed twice for simple sugars (fructose, glucose and sucrose), ascorbic acid (AsA), total and differential organic acids (quinic, oxalic, tartaric, malic, citric and succinic acid) total and differential flavonoids (total quercetins, total kaempferols, total rutins and total naringenins) and carotenoids (β-carotene and trans-lycopene).

2.3.1. Sugars

Simple sugars were extracted according to the following protocol. Briefly, 100 mg of frozen sample were homogenised for 10 min, boiled in 1 mL of 80% ethanol for 30 min, quenched in an ice-bath and centrifuged at 15,000× g for 10 min. The supernatant was filtered through a 0.45 μm polytetrafluoroethylene (PTFE) filter prior to HPLC analysis. The analyses were performed through a Waters E-Alliance HPLC system constituted by a 2695 separations module with quaternary pump, autosampler and a 410 refractive index detector; data were acquired and analysed with Waters Empower software on a PC. A 20 μL aliquot of sample was injected on a Luna NH2 100 Å (250 mm × 4.6 mm, 5 μm particle size) column equipped with a SecurityGuard guard column NH2 (4.0 mm × 3.0 mm) from Phenomenex. The mobile phase was acetonitrile: water (75:25, v/v) with a flow rate of 1 mL·min⁻¹. The analysis lasted for 18 min and the column temperature was set to 30 °C. Peak areas for respective sugars were recorded, and sugar concentration (mg g⁻¹ FW) was calculated using authentic, distinct, and appropriately diluted standards (Sigma-Aldrich, Saint Louis, MO, USA).

2.3.2. Ascorbic Acid

Ascorbic acid (AsA) was determined from an aqueous extract of the tomato fruit (1 g plus 3 mL of 6% metaphosphoric acid in distilled water) homogenised for 30 s using an Ultra-Turrax (IKA, Wilmington, NC, USA), then centrifuged at 1975× g for 15 min. The extraction was repeated twice on the pellet; the supernatant was collected each time and finally made to 10 mL with the extracting solvent. The extracts were filtered through 0.2 μm PTFE filters and analysed using an Ultimate 3000 UPLC system (Thermo Fisher Scientific, Sunnyvale, CA, USA.) at room temperature. A 5 μL sample was injected on a Kinetex (75 mm × 4.6 mm, 100 Å, particle size 2.6 μm) column (Phenomenex, Torrance, CA, USA). The mobile phase constituted 0.02 mol L⁻¹ H₃PO₄ aqueous solution at a flow rate of 0.35 mL min⁻¹. Quantification of AsA was performed at 254 nm using a calibration curve of an authentic chemical standard of ascorbic acid from Sigma-Aldrich (St Louis, MO, USA).

2.3.3. Organic Acids

Quinic, oxalic, tartaric, malic, citric and succinic organic acids were determined from an aqueous extract of tomato fruit (10 g plus 20 mL distilled water), homogenised for 30 s by an Ultra-Turrax, then centrifuged at 4000× g for 20 min, and filtered through 0.45 μm filter.
The extracts were analysed by HPLC at 20 °C, using 0.02 M H₃PO₄ as mobile phase (flow rate 0.8 mL min⁻¹) on a Synergi 4 µm Hydro-RP 80 Å column (250 mm × 4.6 mm, particle size 4.0 µm) and a SecurityGuard guard column (4.0 mm × 3.0 mm) from Phenomenex. Quantification of organic acids was performed at 210 nm through the same HPLC system as for simple sugars analysis equipped with a 2996 photodiode array detector. Organic acids concentration (mg 100 g⁻¹ FW) was calculated using authentic, distinct and appropriately diluted standards (Sigma-Aldrich).

2.3.4. Flavonoids

Flavonoid glycosides were extracted in accordance with a previously described protocol with minor modifications [17]. Briefly, freeze-ground material of tomato pericarp (10 g) was extracted with 1.0 mL of 70% methanol acidified with 0.125% formic acid and mixed immediately for 10 s. The extracts were sonicated for 15 min and filtered through 0.2 µm PTFE filters. A 4 µL aliquot of sample was injected on a Luna C18 (100 mm × 2.0 mm, 2.5 µm particle size) column equipped with a SecurityGuard guard column (3.0 mm × 4.0 mm) from Phenomenex (Torrance, CA, USA). The separations were carried out using a binary gradient of ultrapure water/0.1% formic acid (A) and acetonitrile/0.1% formic acid (B) with a flow rate of 0.2 mL min⁻¹. The initial solvent composition consisted of 97% (v/v) of A and 3% (v/v) of B; decreased linearly to 75% A and 25% B in 4.5 min, 65% A and 35% B in 3.5 min, 10% A and 90% B in 3 min, maintained for 0.5 min and returned to 97% A and 3% B for 1 min. The column was equilibrated to 97% A and 3% B for 5.5 min before the next injection. The analysis lasted for 18 min and the column temperature was set to 40 °C. The system (LTQ XL; Thermo Fisher Scientific) was set up in electrospray negative mode over the range m/z 100–1400. Capillary voltages were set at –5.96 V and the source temperature was 205 °C. Flavonoid glycosides identification was carried out by means of UV spectra, molecular weight and MS-MS fragmentation profile in negative ion mode at a collision energy of 25 eV. Peak areas of quercetin, kaempferol, rutin and naringenin glycosides were obtained by integration at 340 nm. The concentration of flavonoid glycosides was calculated and expressed as mg of Q3G (quercetin 3-glucoside equivalents) 100 g⁻¹ FW.

2.3.5. Carotenoids

Extraction and analysis of carotenoids were carried out on 5 g of fruit pericarp, according to Ishida et al. [18]. Reversed phase-high performance liquid chromatography (RP-HPLC) analysis was performed through Waters E-Alliance HPLC system, as for simple sugars analysis, equipped with a 2996 photodiode array detector; data were acquired and analysed with Waters Empower software. The chromatographic separations were performed at a flow rate of 0.8 mL min⁻¹ and at 0.005 AUFS (Absorbance Units Full Scale) by using a reversed phase, analytical polymeric C30 column (250 mm × 4.6 mm; 3 µm particle diameter; YMC, Wilmington, NC, USA). Results were expressed as µg g⁻¹ of FW. Carotenoid standards (trans-lycopene and β-carotene) used in HPLC analyses were purchased from Sigma Chemical Co (Sigma-Aldrich Company, St. Louis, MO, USA). All solvents used for sample preparation and extractions were of analytical grade, while those for HPLC analysis were of HPLC grade; all were obtained from Merck (Darmstadt, Germany).

2.4. DNA Isolation and Capillary Microsatellite Analysis

Genomic DNA extraction was carried out from 100 mg of young leaves. Leaves were lyophilised in microcentrifuge 2 mL tubes and after adding 2 tungsten balls, tubes were placed in grinding racks and tissue ground to fine powder using Tissue Lyser II (Qiagen, Hilden, Germany) at 30 strokes per second for 30 s. Nucleic acid isolation was then performed using a NucleoSpin Plant II Mini kit (Macherey-Nagel GmbH & Co. KG., Düren, Germany). DNA concentration and quality were measured by absorbance at 260 and 280 nm, respectively, using a UV-Vis spectrophotometer (ND-1000; NanoDrop, Thermo Scientific, Wilmington, DE, USA). DNA solution was then diluted to a working concentra-
tion with distilled water. Genetic diversity was assessed using 10 SSRs representatives of various repeat classes [19,20] (Table 1). For all markers, the forward primers were 5’-end labelled with the fluorescent dye markers FAM; the reverse primers were not labelled. PCR amplifications were performed in 15 µL reactions containing 40 ng of template DNA, 0.2 µM of each forward and reverse primer, 0.2 mM of each dNTP, 1.5 Units of Dream Taq polymerase (Fermentas, Waltham, MA, USA). The reactions were amplified using a C-1000 Touch Thermal Cycler (Bio-Rad, Hercules, CA, USA). Amplification consisted of an initial denaturation at 95 °C for 3 min, followed 35 cycles of 94 °C for 45 sec, specific T° of annealing for 45 sec, and 72 °C for 1 min 30 sec, a final extension of 72 °C for 10 min and soaking at 8 °C. After dilution 1:50, 1 µL of the fluorescent labelled amplicons were mixed with 0.3 µL of GeneScan-600 LIZ (GS 600) size standard (Thermo Fisher Scientific, Waltham, MA, USA) and 13.7 µL of Hi-Di Formamide (Thermo Fisher Scientific, Waltham, MA, USA). Capillary electrophoresis was performed in a SeqStudio™ Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). Signal peak height and allele sizes were calculated on the GeneMapper™ Software v.6 based on GS 600 molecular weight standard.

| Marker Name | Forward | Reverse | Annealing °C | Size (bp) | Reference |
|-------------|---------|---------|--------------|----------|-----------|
| TCI C-419   | TGACCAACATAATAATGATATGCC | AGAAACAATCTAGTGGTTCCATCACC | 55 °C | 444 | [19] |
| LE aat002   | GGGAAGAGATGAGCTAGAGCATAG | CTCCTCCTCAGATGCTCCTCCTTC | 53 °C | 102–105 | [20] |
| LE aat007   | CAACACCATATGAGAGGAGG | TACATTCTCTCCTCCATCATAG | 53 °C | 95–98 | [20] |
| LE20852     | CTGTTTACCTACAAGAAGGCTG | ACTTTAACATTATATCGACAGCAG | 53 °C | 161–167 | [20] |
| LE21085     | CATTGTTATATTTTATTTTGATTCGTCCTT | ACAAAAAAGGAGAACGCCATACA | 50 °C | 102–103 | [20] |
| LEeta003    | GCTCTGTCCCTTACAATAAGATGATCCTCC | CAATGCTGGGACAGAAGATTTAAGT | 53 °C | 103–108 | [20] |
| LEeta002    | ACCTTTGCCGTCCTGCGCA | AACTTTAACATTATAGCTACAGTGA | 55 °C | 99–105 | [20] |
| LEcct001    | TCCAAATTGCAAGGACCCCTTC | CGGAAAACTTTGGTACAGTACAGTA | 53 °C | 98–107 | [20] |
| LEcct001    | CCTCCTCTAACCTTACATATTTC | CACTTGCTATGAGTCTACAGGC | 55 °C | 90–91 | [20] |
| LEeta015    | ATATGCATGGACAAATCTCGAGGG | CTCCGCGATCAAATATGATCTCAG | 55 °C | 101–111 | [20] |

2.5. High-Resolution Melting Analysis

High resolution melting (HRM) analysis was performed to corroborate capillary fragment separation. Not labelled forward and reverse primers were used. For each HRM assay, three replicates were considered. PCRs have been performed in a volume of 10 µL per reaction in a 96-well Bio-Rad CFX 96 RealTime PCR System (Bio-Rad, Inc., Hercules, CA, USA). Reaction mixtures included 5 µL of 2× Precision Melt Supermix (Bio-Rad) (final concentration 1×), 0.4 µL of each primer (final concentration 200 nM) and 2.5 µL (conc 15 ng/µL) of genomic DNA. Sterile H2O to final 10 µL volume. A negative control was included in each assay. Amplification protocol started with 95 °C for 2 min followed by 40 cycles of 95 °C for 15 s and 58 °C for 30 s. The melting curve was obtained as follows: an initial heteroduplex formation step of 95 °C for 30 s and 64 °C for 3 min, then the HRM rising from 65 °C to 95 °C with increments of 0.2 °C/cycle every 10 second. Melting profiles and genotype discrimination were performed with the Precision Melt Analysis™ Software (Bio-Rad), which assigns species to determined clusters calculating the percent of confidence that provides the threshold that a given sample is properly assigned with the cluster. We considered values >95% as threshold, for group assignment.

2.6. Phenotypic Data Analysis

All traits were subjected to multivariate analysis of variance (MANOVA) using IBM SPSS Statistics, version 25.0 (IBM: CorpArmonk, NY, USA) to determine the overall differences between varietal groups and landraces. Wilk’s lambda (unexplained variance rate) was applied for testing the significance of MANOVA. The normality of the residuals and residuals standardised was determined using both Kolmogorov–Smirnov and Shapiro–Wilk’s tests. The homogeneity of variances was checked by computing Bartlett’s test in R version 3.0.2 (R Development Core Team) [21]. For traits showing homogeneity of
variances means were compared using Tukey’s honestly significant difference test ($p \leq 0.05$) using JMP, version 7.0 (SAS Institute, Cary, NC, USA) in accordance with a completely randomised design. If the homogeneity of variances was violated, we separated groups by Games-Howell’s post hoc test to reduce the Type I error in the case of heteroscedasticity. Connecting letters report from a Games-Howell’ test was obtained using the Games-Howell Test v.1.2. jmpaddin implemented in JMP version 16 (SAS Institute, Cary, NC, USA). Coefficient of variation (CV) in percentage was expressed as the ratio of the standard deviation to the mean value multiplied by 100. Correlation test was used to compare the distribution of morphological, chemical and biochemical traits. Pearson analysis was carried out testing at $p < 0.01$. Principal component analysis (PCA) was performed using the computer package XLSTAT 2012.1. Correlogram was constructed and visualised using the Corrplot package implemented in R v.3.0.2 [21].

2.7. Genetics Data Analysis

For microsatellite several statistical parameter were calculated as following: (I) Polymorphic Information Content (PIC) was estimated by determining the allele frequency per locus using the formula $\text{PIC}_m = 2 f_m (1 - f_m)$, where $\text{PIC}_m$ is the polymorphic information content of marker $m$, and $f_m$ is the frequency of the polymorphism present at given locus, and $(1 - f_m)$ is the frequency of absent polymorphism for each locus; (II) effective multiplex ratio (EMR) is defined as the product of total polymorphic loci ($p_l$) and the number ($n$) of polymorphic loci for an individual assay ($p_l/t_l$); (III) marker index (MI) is the product $\text{PIC} \times \text{EMR}$ and estimate the overall efficiency of the molecular marker assay; (IV) resolving power (RP) defined as $\text{RP} = \Sigma I_b$, in which $I_b$ represents the polymorphism informativeness that takes the values of $1 - [2 (0.5 - p)]$, $p$ being the proportion of each genotype containing the polymorphism. Genetic structure was inferred using the Bayesian-based cluster algorithm implemented in the software STRUCTURE version 2.3.4 [22]. We used Admixture model analysis, assuming correlation among alleles frequencies and Markov Chain Monte Carlo (MCMC) method for allele frequencies estimation and identification of best number of population (K). Runs were performed 100,000 burn-in cycles and 100,000 MCMC iterations, number of K from 1 to 10 with five independent runs for each K. The most probable numbers of K were determined using Structure Harvester [23]. Accessions were considered to belong to a specific sub-population if its membership coefficient ($q_i$) was $\geq 0.50$, whereas the genotypes with $q_i$ lower than 0.5 at each assigned K were considered as admixed. A phylogenetic tree was drawn using the Maximum Likelihood method and the Tamura-Nei model with 500 bootstraps. Analyses were conducted in MEGA X software [24].

3. Results

3.1. Phenotypic Variation of ‘San Marzano’ and ‘Re Fiascone’ Groups

In Table 2 are reported the MANOVA analysis considering as factors: (a) the cultivar groups ‘San Marzano’ and ‘Re Fiascone’; (b) the 21 studied accessions. In both instances, all statistical tests were significant.

Table 2. MANOVA analysis considering as ‘factor’ the cultivar groups (‘Re Fiascone’ and ‘San Marzano’) and the 21 studied accessions (factor = genotype).

| Effect       | Test Statistic | Value | F  | df Hypothesis | df Error | Sig. ($p$) | Value | F  | df Hypothesis | df Error | Sig. ($p$) |
|--------------|----------------|-------|----|---------------|----------|------------|-------|----|---------------|----------|------------|
| Category     | Wilks’s lambda | 0.06  | 20.02 | 26            | 36       | <0.001     | 0.00  | 8.81 | 520           | 353      | <0.001     |

In Table 3, the MANOVA results, and the mean, minimum, maximum, coefficient of variation (CV) for each trait specify the performance of ‘San Marzano’ and ‘Re Fiascone’ cultivars.
**Highly significant differences among groups (p < 0.001)** were found for fruit morphological traits excluding fruit shape, and among biochemical compounds for malic and succinic acids. Less stringent differences (p < 0.01) were found for soluble solids, acidity and citric acid. The remaining traits did not show significant differences among cultivar groups. In average, ‘San Marzano’ cultivars are characterised by fruits mostly having a cylindrical shape, with less weight and greater thickness of the pericarp whereas, ‘Re Fiascone’ included fruits mostly rounded or slightly elongated (Figure 2). The soluble solid content was in average lower than five in both groups with the average higher content found in ‘San Marzano’ cultivars. The same trend was observed for total acidity whereas, pH was in average higher considering both Kolmogorov–Smirnov and Shapiro–Wilk test (Table S1). In total, five metabolic traits had a significant p-value within both tests including pH, total acidity, sucrose, quinic acid and trans-lycopene. For all traits, the normality of residuals has been calculated with less weight and greater thickness of the pericarp and tartaric acids and three using only one of the two tests (trans lycopene, citric acid and total naringenins). The results of homogeneity test of variance using Bartlett’s test highly significant differences among groups (p < 0.001) were found for soluble solids, acidity and citric acid. The remaining traits did not show significant differences among cultivar groups. In average, ‘San Marzano’ cultivars are characterised by fruits mostly having a cylindrical shape, with less weight and greater thickness of the pericarp whereas, ‘Re Fiascone’ included fruits mostly rounded or slightly elongated (Figure 2). The soluble solid content was in average lower than five in both groups with the average higher content found in ‘San Marzano’ cultivars. The same trend was observed for total acidity whereas, pH was in average higher considering both Kolmogorov–Smirnov and Shapiro–Wilk test (Table S1). In total, five metabolic traits had a significant p-value within both tests including pH, total acidity, sucrose, quinic acid and trans-lycopene. For all traits, the normality of residuals has been calculated with less weight and greater thickness of the pericarp and tartaric acids and three using only one of the two tests (trans lycopene, citric acid and total naringenins). The results of homogeneity test of variance using Bartlett’s test.
are in Table S2. Eleven traits rejected the null hypothesis showing a non-homogeneity of variances. Post-hoc analysis for traits showing both homogeneity and non-homogeneity of variance has been performed according to Tukey’s (Table S3) and Games-Howell’s tests (Table S4), respectively. Mean and standard deviation for the morphological, chemical and phytochemical traits analysed in the 21 tomato accessions are reported in Table S3.

3.2. Morphological and Chemical Traits Performances

In the studied set, the fruit weight was in average 60.93 g with the peak of 82.44 g in ‘FSCF’ and the lowest value of 27.38 g in ‘Smec 20’ (Table S3). Fruit dimensions differ according to the cultivar group with the highest length of 74.11 mm in ‘SML’ and the highest width of 52.87 mm in ‘FSC’. These two genotypes showed also the highest and lowest fruit shape index (Table S3) indicating cylindrical and round-shaped fruits, respectively. All berries showed a consistent pericarp with an average thickness of 6.43 mm and values above 8 mm in ‘FSCF’, ‘FSC3’, ‘SML’ and ‘SMF7’. Among the chemical traits, the soluble solid content was in average lower than five with a peak of 5.97 °Brix in ‘SMF6’ and the lowest values of 3.97 °Brix in ‘KIRna’, both belonging to ‘San Marzano’ (Table S3). pH was instead similar among genotypes with values above 4.00 except than ‘SMm’ and ‘KIRna’ showing 3.22 and 3.97, respectively. Total acidity was instead highly variable with an average of 0.42% ranging from 0.62% for ‘SMF6’ to 0.32% in ‘FSCF’, ‘FSC1’ and ‘FSL’.

Figure 2. Details of fruit morphology of the tomato accessions considered in the study. In orange and red labels are indicated ‘Re Fiascone’ and ‘San Marzano’ landraces, respectively.
3.3. Bioactive Compounds

Glucose, fructose and total simple sugars in the twenty-one tomato genotypes studied ranged from 3.07 to 8.31, 6.49 to 13.37 and 9.59 and 21.83 mg g\(^{-1}\) FW, respectively, with...
the highest amounts being measured in ‘FST’ and the lowest ones in genotype ‘FSC4’ (Table S3). Sucrose content was undetectable in some genotypes and no significant differences were evidenced in the remaining ones. Seven out of 21 genotypes (‘FSC1’, ‘FSC2’, ‘FSloc’, ‘KIRsa’, ‘KIRna’, ‘SML’ and ‘FST’) analysed showed glucose, fructose and total simple sugars concentration higher than the average concentrations (5.14, 9.27 and 14.49 mg g\(^{-1}\) FW, respectively).

The highest content of ascorbic acid was found in genotype ‘FSC1’ (22.48 mg 100 g\(^{-1}\) FW) and the lowest in genotypes ‘SMn’ and ‘SMF6’ (10.17 and 10.32 mg 100 g\(^{-1}\) FW, respectively). Eleven out of 21 genotypes analysed showed AsA concentrations higher than the total average concentration of 15.64 mg 100 g\(^{-1}\) FW.

As revealed by the large number of significant differences found, organic acids varied considerably among the accessions studied with values ranging from 209.97–678.13 mg 100 g\(^{-1}\) FW. Citric acid was the predominant organic acid being from 1.2 (FSC4) up to 10-fold (‘SMFT’) higher with respect to malic acid. Five accessions showed instead a higher value of malic acid with the highest ratio MAL/CTA in ‘FSloc’ (Figure 3b). Overall, eleven out of 21 genotypes studied displayed total organic acids amount higher than the total average concentration of 456.88 mg 100 g\(^{-1}\) FW.

Flavonoid glycosides, as previously reported by Moco et al. [25], were detected and identified based on MS-MS fragmentation and UV spectra. Quercetin was only found as glycoside forms. Three quercetin glycosides were tentatively identified and quantified: quercetin-dihexose-deoxyhexose (m/z = 771.17), quercetin-hexose-deoxyhexose-pentose (m/z = 741.17) and quercetin-hexose-deoxyhexose-pentose-p-coumaric acid (m/z = 887.11). Two major kaempferol glycosides were putatively identified: kaempferol-3-rutinoside (m/z = 593.10) and kaempferol-3-(2-pentose,6-rhamnose)-hexose (m/z = 725.08) as previously described by Gómez-Romero et al. [26]. Rutin (m/z = 609.17) and rutin-hexose (m/z = 771.07) were tentatively identified as reported by Moco et al. [25]. Two naringenins were tentatively identified: naringenin chalcone (m/z = 271.25) and naringenin chalcone-dihexose (m/z = 595.17) as reported by Slimestad et al. [27].

Quercetins, kaempferols, rutins and total flavonoids ranged from 0.84–7.54, 0.31–3.63, 0.69–9.84 and 2.20–23.84 mg Q3G 100 g\(^{-1}\) FW in ‘FSC2’ and ‘FSC3’, respectively; naringenins ranged from 0.31–3.43 mg Q3G 100 g\(^{-1}\) FW in ‘FSC’ and ‘FST’, respectively. Rutins and quercetins were the most represented flavonoids with a ratio between the two compounds near to one except that for the two ‘San Marzano’ accessions ‘SML’ and ‘Sme20’ having a ratio RUT/QUE ~2 (Figure 3c). For each of the flavonoids studied eight or nine genotypes showed concentrations higher than the total average concentrations; interestingly, the genotypes ‘FSC3’ and ‘KIRsa’ displayed, for all the flavonoids studied, amount higher than the total average concentration (3.43, 1.45, 3.72, 1.64 and 10.24 mg Q3G 100 g\(^{-1}\) FW, respectively) (Table S3).

β-carotene in the twenty-one tomato genotypes studied ranged from 3.55 to 16.29 µg g\(^{-1}\) FW with the highest amount being measured in ‘FSloc’ and the lowest one in genotype ‘FSC’. Nine out of 21 genotypes analysed showed β-carotene concentration higher than the total average concentration of 7.03 µg g\(^{-1}\) FW. Trans-lycopene ranged from 27.28 to 112.82 µg g\(^{-1}\) FW; the highest amount was measured in ‘SML’ whereas low amounts were detected in ‘FSC4’, ‘FSCF’, ‘FSC’, ‘SMm’ and ‘FSC2’. Eight for β-carotene and eleven for trans-lycopene out of 21 genotypes studied evidenced concentrations higher than the total average concentrations (7.03 and 65.37 µg g\(^{-1}\) FW, respectively); interestingly, eight genotypes (‘SMs’, ‘FSC1’, ‘FSloc’, ‘FSL’, ‘Arkos’, ‘KIRna’, ‘SML’, ‘SMF6’) showed either β-carotene or trans-lycopene concentrations higher than the total average concentrations (Table S3).

3.4. Multivariate Analysis and Correlations between Traits

The PCA in the first two dimensions, for the morphological, chemical and biochemical traits, revealed 36.21% of the total variance (Figure 4). Accessions of the two cultivar groups and traits were evenly distributed in both negative and positive axes of the graph. The first
component which explained the 19.16% of the total variance was positively correlated with morphological traits except FS, flavonoids, soluble solids and total acidity, and negatively correlated with sugars, organic acids except oxalic acid, β-carotene, trans-lycopene and ascorbic acid. The second component which explained the 17.05% of the total variance was positively correlated with all flavonoids, trans-lycopene, β-carotene and most organic acids and negatively correlated with sugars and ascorbic acid. Morpho-agronomic and chemical traits were interspersed in both the negative and positive parts of the second component. ‘San Marzano’ accessions tended to cluster mostly in the positive axis of PC2 except for SML and SMF7. On the contrary, ‘Re Fiascone’ genotypes were mostly in the negative part of PC2 apart from ‘Arkos’ and ‘FSloc’. Overall, ‘Re Fiascone’ cultivars showed the highest variability for the traits considered.

Rutins and citric acid were the main factors discriminating the genotypes under study accounting for 13.38% and 12.86% of the total variation of the first and second component, respectively (Table S5). Significant correlation between pairs of variables mostly occurred among the same group of traits (Figure 5). Between morphological, chemical and biochemical traits, we found negative correlation of fruit weight with total acidity and among organic acids, citric acid and positive correlation of pericarp thickness with trans-lycopene. β-carotene was positively correlated with malic acid and trans-lycopene. Ascorbic acid was positively correlated with fructose and tartaric acid. Total acidity was highly correlated with positive sign with citric and succinic acid whereas, for the other organic acids the correlation was very weak.

**Figure 4.** Loading plot of the first (PC1) and second (PC2) principal components showing the variation for 27 morpho-agronomic and phytochemical traits scored in 21 tomato accessions. ‘Re Fiascone’ and ‘San Marzano’ are indicated with different coloured symbols. On the right are indicated the distribution of the traits scored. The direction and distance from the centre of the biplot indicate how each OTU contributes to the first two components. The different category of traits is indicated using different colour codes as following as indicated in the legenda at the bottom. Beta-carotene and trans-lycopene are indicated with orange and red rhombus, respectively. Ascorbate is indicated by black, ‘x’ Trait acronyms are listed in Table 3.
Agronomy 2022, 12, x FOR PEER REVIEW...omomorphic. Overall, LEaat002 resulted to be the best marker for the discrimination of the accessions using both essays.

3.5. Genetic Diversity

For genetic diversity analysis 10 microsatellites comprising various repeat classes were selected for their ability to generate polymorphism based on previous information (Table 1) [11,19,20]. Considering the SSR fragment analysis by capillary electrophoresis (CE-SSR), we detected 36 alleles for an average of 3.6 alleles per locus (Table 3). The number of alleles at each locus varied from two for LE21085 to seven for LEta015. Only the marker TCI C-419 was monomorphic. Among the 10 SSR used, the mean PIC was 0.33, with values ranging from 0.20 for LE20592 to 0.30 for LEtat002. EMR ranged from two for LE21085 to seven for Leta015. MI ranged from 0.27 for LEta015 to 3.11 for LEaat002. RP ranged from 0.22 for LE20592 to 1.86 for LE21085. Considering the SSR analysis by HRM (HRM-SSR) we detected similar number of total alleles based on melting profiles being 37 for an average of 3.7 alleles per locus (Table 4).

The range of alleles found at each locus was lower varying from three for LE21085, LEaat002 and LEta015 to six for LE20592 (Figure 6). Same trend was observed for the average PIC that was 0.31, with values ranging from 0.23 for LE20592 to 0.44 for LEtat002. EMR ranged from three for LE21085, LEtat002, Leta015 to six for LE20592. MI ranged from 0.73 for LEta015 to 1.60 for LEaat002. The RP was in average lower showing the maximum value of 0.67 in LEtat002. The marker TCI C-419 was confirmed monomorphic. Overall, LEaat002 resulted to be the best marker for the discrimination of the accessions using both essays.
The two minor clusters comprised seven ‘Re Fiascone’ and two ‘San Marzano’ accessions, highlighting the existing genetic diversity according to cut off confidence value of 95%. From marker statistics each cluster is considered as a separate allele. Partitioning of the collection based on polymorphism obtained by combination of CE and HRM data and independent STRUCTURE imputations at a range of K-values from 1 to 10 and Evanno’s test (Table S6) showed peaks at K = 3, K = 7 and K = 9; the latter with the maximum delta K, suggesting that the most likely number of subpopulations included nine subclusters (Figure S1). At K = 3 (Figure 7a), a division according to ‘Re Fiascone’ and ‘San Marzano’ was evident. The two minor clusters comprised seven ‘Re Fiascone’ and two ‘San Marzano’ accessions, highlighting the existing genetic diversity according in the two cultivar groups. The larger cluster composed of 12 genotypes, included all the

Table 4. Number of loci, polymorphic index content value (PIC), resolving power (RP), effective multiple ratio (EMR) and marker index (MI) obtained by the SSRs used in the present work using capillary electrophoresis (CE) and high resolution melting (HRM) analysis.

| Marker Name | Alleles | CE-SSR | HRM-SSR |
|-------------|---------|--------|---------|
|             |         | PIC    | RP      | EMR    | MI   | Alleles | PIC    | RP      | EMR    | MI |
| TCI C-419   | 1       | 0.00   | 0.00   | 0.00   | 0.00 | 1       | 0.00   | 0.00   | 0.00   | 0.00 |
| LEaat002    | 4       | 0.50   | 0.98   | 5.00   | 2.00 | 5       | 0.32   | 0.40   | 5.00   | 1.60 |
| LEaat007    | 3       | 0.43   | 0.63   | 3.00   | 1.30 | 4       | 0.38   | 0.50   | 4.00   | 1.50 |
| LE20592     | 5       | 0.20   | 0.22   | 6.00   | 1.20 | 6       | 0.23   | 0.27   | 6.00   | 1.40 |
| LE21085     | 2       | 0.13   | 1.86   | 2.00   | 0.27 | 3       | 0.38   | 0.51   | 3.00   | 1.14 |
| LETat003    | 4       | 0.39   | 0.52   | 4.00   | 1.55 | 4       | 0.36   | 0.48   | 4.00   | 1.45 |
| LETat002    | 4       | 0.44   | 0.64   | 4.00   | 1.74 | 3       | 0.44   | 0.67   | 3.00   | 1.33 |
| LEctt001    | 3       | 0.42   | 0.60   | 3.00   | 1.26 | 4       | 0.38   | 0.50   | 4.00   | 1.50 |
| LEctt001    | 3       | 0.31   | 0.38   | 3.00   | 0.93 | 4       | 0.36   | 0.48   | 4.00   | 1.45 |
| LEta015     | 7       | 0.44   | 0.67   | 7.00   | 3.11 | 3       | 0.24   | 0.29   | 3.00   | 0.73 |
| Total       | 36.00   | 3.26   | 6.51   | 37.00  | 13.36| 37.00   | 3.10   | 4.08   | 36.00  | 12.11|
| Mean        | 3.60    | 0.33   | 0.65   | 3.70   | 1.34 | 3.70    | 0.31   | 0.41   | 3.60   | 1.21 |

Figure 6. Genotyping of 21 tomato accessions with markers showing low and high polymorphisms based on the resolution melting (HRM) profiles. Curved with different colours correspond to clusters according to cut off confidence value of 95%. From marker statistics each cluster is considered as a separate allele.
‘San Marzano’ types and the three ‘Re Fiascone’ ‘FSC1’, ‘FSloc’ and ‘FST’. By increasing K, the principal ‘Re Fiascone’ and ‘San Marzano’ cluster were confirmed evidencing a certain level of admixture for ‘FCSF’, ‘FSC2’ and ‘FSC3’. The major cluster was instead split in more subgroups separating ‘SMF7’, ‘Smec20’ and ‘Smec 24’ from the rest whereas, the remaining accessions showed a different degree of admixture (Figure 7a).

The maximum likelihood-based phylogenetic tree based on Tamura-Nei model divided the collection into two major branches (Figure 7b) confirming grouping according to the population STRUCTURE. The dendrogram better highlighted the presence of subgroups based on varietal types. The first branch (a) grouped the two minor clusters of ‘Re Fiascone’ and ‘San Marzano’ highlighting the strong similarity for the two Kiros genotypes (‘KIRna’ and ‘KIRsa’) as well as for ‘FSC’ and ‘FSC3’. The second branch (b) highlighted the strong similarity of the accessions of the different cultivar groups including ‘SMs’ and ‘SMn’, ‘FSC1’ and ‘FSloc’, ‘Smec 20’ and ‘Smec 24’.

**Figure 7.** Genetic diversity of the collection studied based on polymorphisms detected with both capillary electrophoresis and high resolution melting using ten microsatellite markers. (a) STRUCTURE analysis of three (K = 3), seven (K = 7) and nine (K = 9) cluster. The vertical bars indicate the membership coefficient for each individual. Coloured blocks correspond to the different clusters. (b) Circular diagram showing maximum likelihood-based phylogenetic analysis based on Tamura-Nei model.

**4. Discussion**

Modern agriculture, and development of cultivars for high input cultivation systems, has narrowed the diversity in many crops [28,29]. Rediscovery of ancient varieties selected to specific environments represent a fundamental strategy for preserving biodiversity and promoting new germplasm for meeting the needs of sustainable agriculture. In fact, by prospering in marginal areas, landraces have been shaped to be adapted across years to contrasting conditions in their original territory being, therefore, a potential source of unique and undiscovered alleles of agricultural interest [30,31]. In addition, these plants often offer a high content of phytochemicals beneficial for human health, underlying variegated organoleptic profiles, and conferring defence mechanisms against different types of stresses [32,33]. In fact, previous investigation highlight tomato landraces as promising sources of resistance/tolerance against infections of late blight (*Phytophthora infestans*), early blight (*Alternaria solani*) and Septoria leaf spot (*Septoria lycopersici*) [33].

In this study, we analysed the diversity of two outstanding landraces selected in Campania, a region with a strong vocation for the cultivation of tomatoes and located...
in its secondary diversity centre. The panel of accessions showed a wide diversity for the traits analysed, although only a few well enlightened the diversity between ‘San Marzano’ and ‘Re Fiascone’ groups. In vegetables, tomatoes are the primary source of lycopene, a compound providing the red colour to fruits and representing 80–90% of total carotenoids whereas, β-carotene, the precursor of provitamin A, is the second most important carotenoid representing ~10% of the total [34]. Both could provide added value due to their nutritional and antioxidant properties. We found in the collection studied, a good source of trans-lycopene compared to previous studies on Spanish landraces [35], cherry tomato varieties spread in Italy [36] and cultivars grown in Eastern Europe [37], although the level was in average lower than high-lycopene genotypes [38]. Moreover, we identified some accessions reaching outstanding values of β-carotene, (e.g., ‘FSlloc’, 16.29 µg g⁻¹ FW) even compared to high pigment and high-lycopene tomatoes [35,38] (average ≤ 13 µg g⁻¹ FW) and hybrids [37], while vitamin C agreed with the previous studies [34]. Beyond genotype it must be taken into consideration that several factors could influence the accumulation of these compounds including the environment of cultivation and ripening stage [39]. Indeed, it has been observed that the cultivation in the open field could impact positively the content of lycopene [40], therefore it is likely that these accessions could provide a higher lycopene content if grown in the natural environment. Overall, we found accessions that well suited for human consumption, as an example, just 100 g of fresh fruit of ‘SML’ provide 45% of the recommended daily lycopene intake (25 mg) or 40% of the required vitamin C dosage (60 mg) [41,42].

Tomato is among the most important providers of phenols among vegetables [43], as previously observed we found rutins and quercetins the most represented flavonoids [39,44]. Rutin is considered the flavonoid with the highest contribution to the hydrophilic antioxidant activity of tomatoes [39], outstanding levels of this compound were detected in accessions of both ‘San Marzano’ and ‘Re Fiascone’ groups. In particular, ‘SMm’ (5.96 mg Q3G 100 g⁻¹ FW) and ‘FSC3’ (9.84 mg Q3G 100 g⁻¹ FW) exhibited higher content compared with elite cultivars for fresh and processing destination showing values up to 43.31 mg/kg and 26.56 mg/kg FW, respectively [39].

We also investigated the content of sugars and organic acids, key components underlying the taste and flavour of tomato, and therefore responsible for the overall quality and consumer acceptance [45]. Previous attempts on ‘San Marzano’ cultivars [46] report average higher content for both fructose (1.4 g/100 g) and glucose (1.2 g/100 g) with respect to our findings. Similar levels were found only for fructose in the two ‘KIRna’ and ‘KIRsa’ genotypes. Sucrose was confirmed to be present in trace [16]. On the contrary we found high levels of organic acids with respect to previous reports [47].

The phenotypic characterisation has been strengthened by marker analysis. The HRM approach for genotyping resulted to be more powerful in polymorphisms detection in most of the markers. The ability of HRM to identify melting curves based on composition and position of single nucleotide polymorphism differences (SNP), makes this method more sensitive compared to CE-SSR. Our results agree with previous works combining the two approaches [48]. The genetic diversity analysis showed a level of differentiation between ‘San Marzano’ and ‘Re Fiascone’ accessions. The optimal number of K clusters that is unusual in low population size as in this study could be explained by alleles highly conserved in determined accessions, but also by the high uniformity of some genotypes compared to the rest. This is highlighted by the increase of admixed accessions from K = 3 to K = 9 while others remain uniform. In addition, the phylogenetic relationships revealed those very close related accessions. The clustering between the two ‘San Marzano’ and ‘Re Fiascone’ groups and the relationships between the sampled landraces could be explained by both restricted gene flow between the different rural areas and also from a common selection process by farmers. Sharing of common ancestors could be the reason for accessions intermingled between the two groups. Further investigations, that increase the depth and the throughput of genotyping (e.g., use of arrays or sequence-based genotyping) could provide more insight of the history and the population structure of these ancient
varieties as done in long shelf life ‘da serbo’ landraces [49]. The integration of bioactive compounds levels in multivariate analysis, allowed to discriminate a well-performing set of genetically distinct cultivars.

These results demonstrated how traditional varieties could be used to improve the quality of modern cultivars, suggesting furthermore a pyramiding breeding strategy to combine different traits in a single ideotype. Efforts to promote these cultivars in sharing economy strategies are ongoing. For ‘Re Fiascone’ a crowdfunding campaign for seed recovery and cooperation between custodian farmers has been recently launched towards the development of agribusiness activities that boost its commercialisation [50]. ‘San Marzano’ after its decline due to the diffusion of commercial hybrids possessing similar fruit morphology has been rediscovered owing to the approval of PDO certification. Regional ongoing projects are promoting in situ cultivation. Further investigations towards the identification of disease tolerances to major pests and pathogens will be performed with the aim of broadening the information on the potentialities of these landraces for genetic improvement.

5. Conclusions

In the changing societal, agricultural and environmental scenario, traditional landraces are being rediscovered by the community that is more aware of sustainable productions, healthy products and links with the territory, thus representing a pillar for promoting local microeconomies activities. The approach pursued highlights the potential of unexplored germplasm, allowing the selection of specific genotypes to promote not only the marketing of the product but also for exploring genetic improvement for quality-linked traits. Based on the information retrieved from this study, it is possible to select a set to investigate in further sensory panels investigations, providing more insight into the preference of consumers.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy12010018/s1. Figure S1: Evaluation plots of the best grouping number (K) of the Bayesian clustering analysis using the Evanno’s method. Table S1: Test of normality of the residuals and standardized residuals. In bold traits violating the normality of residuals. Table S2: Bartlett’s test for homogeneity of variance of the traits tested in 21 tomato landraces. In bold traits rejecting the null hypothesis. Table S3: Mean values and standard deviations for agronomic and biochemical traits evaluated in 21 tomato accessions. In bold traits exhibiting homogeneity of variance according to Games–Howell post-hoc test for traits showing non-homogeneity of variance. In each column, values indicate differences between genotypes (I–J) +/− standard error. Significance a p < 0.05, p < 0.01 and p < 0.001 are indicated in bold with *, ** and ***, respectively. Table S5: Variable contribution (VarPC), Eigenvectors (Egv) and correlation coefficient (CorrPC) for morphological, chemical, and phytochemical traits in the first two principal components. Table S6: Membership of 21 tomato accessions to subpopulations developed at K = 3, K = 7 and K = 9 according to STRUCTURE analysis.

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