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Diversity assessment in the Portuguese genebank collection of tomato (*Solanum lycopersicum* L.) accessions: Insights for breeding and sustainable conservation

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ABSTRACT

Tomato germplasm is source of variability used by plant breeders to develop improved cultivars and part of the natural heritage to be preserved. In this study, the diversity among twenty Portuguese tomato accessions was evaluated, considering morphological and agronomic descriptors, molecular data, and compositional aspects. The tomato accessions captured 77.5% of the total 19 alleles detected. Important information on the selection of SSR *loci* in diversity analysis for the tomato genotypes was provided. The studied genotypes were rich in carbohydrates and source of ascorbic acid. Citric acid and palmitic, linoleic, oleic, stearic, and α-linolenic acids were the major organic and fatty acids, respectively. The four tocopherol isoforms were detected and α-tocopherol was the most abundant. When correlating molecular and compositional data, it seemed that the greater the heterozygosity, the greater the compositional variance. However, despite the genetic distance that characterized these accessions, some were chemically similar. Overall, the genetic differentiation among genotypes provided useful information for parental selection in breeding and other genetic studies.

1. Introduction

Biodiversity conservation is fundamental to sustain population growth, ensure food security, and improve global nutrition, promoting well-being. Conservation programmes and strategies should focus on the ecology and socio-economy of diverse agroecosystems and low-input cropping systems, providing knowledge and awareness about ecosystems services, agricultural research and innovation, efficiency in resource management, production stability, minimal environmental impact, resilience to changes and adaptation to local conditions (Bommarco et al., 2018).

Genetic diversity of crops, landraces, wild relatives, and other wild plant species provides the basic building blocks to improve the productivity, resilience, and nutritional composition of food (Roa et al., 2016). Landraces and traditional produces have been progressively displaced by biotechnologically improved varieties and crops. Thus, germplasm collections held in genebanks are of great importance because they preserve diversity avoiding genetic erosion, as well as the knowledge and practices linked with wild species, landraces, farmer varieties, for instance. However, there is an important debate concerning plant breeding and genetic diversity. Several researchers have shown how genetic diversity is reduced by continued selection in the breeding germplasm, but some others suggest that diversity might increase by introgression of alleles from wild relatives, which compensates genetic loss by inbreeding and selection, as studied in tomato and grain commercial varieties (Schouten et al., 2019).

Much effort has been made to characterize and promote the use of the accessions (*i.e.*, individual samples of each species or variety

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preserved) held in genebanks, since these resources have profound implications for continuous progress in plant breeding as well as for food sovereignty and security within a context of climate change, sustainability, and diminishing crop diversity *in situ*. Therefore, essential information about germplasm accessions must be thoroughly catalogued (*e.g.*, agronomic, biochemical, genomic, and other traits linked to genebank accessions) and publicly available (Anglin et al., 2018).

The tomato (*Solanum lycopersicum* L.) belongs to the Solanaceae botanical family, the third most economically important in Plant Kingdom after Poaceae and Fabaceae (Ghatak et al., 2017), which has played a central role in human nutrition since primordial times. Tomato stands out among horticultural crops, globally, the most versatile and important, both for fresh and processed consumption, and of great nutritional relevance (Pinela et al., 2016, 2019). Nowadays, the consumption of tomato (fresh and processed) is still growing, having been produced about 189 million tons in 2021 worldwide. More than a half of this production comes from Asia (~63%), followed by the Americas and Europe. The European Union (UE-27) held 9.5% of the world production and Italy was the member state with the highest production (~37.1%). Portugal was the third largest European producer of tomato with approximately 1.7 million tons, corresponding to 9.7% of the total registered in the EU (FAOSTAT, 2023). Considering the production of tomatoes for fresh consumption in the EU-27 (which correspond to 39.6% of the overall tomato production), Spain was the main producer in 2021 (23.6%) and Portugal was in the ninth position (European Commission, 2022). Nevertheless, in Portugal, fresh tomato was the horticultural crop with the highest production (157 thousand tons) in 2021 (INE 2021), representing an increase of ~25% compared to 2020 (INE, 2021). Portuguese statistical data does not specify if local farmer varieties and tomato landraces account to this number. Some studies conducted in the Iberian Peninsula and the Mediterranean region highlight the importance of farmer varieties and tomato landraces for local consumers (Botelho et al., 2014; Conesa et al., 2020; Lázaro, 2018). For instance, surveys carried out in the north and center regions of Portugal showed that traditional tomato varieties were rated higher than foreign cultivars and that consumers were willing to pay 35% more for these traditional varieties (Botelho et al., 2014). Promoting the preservation of traditional Portuguese varieties of tomatoes requires the best information about their characteristics (including agronomical, morphological, chemical, molecular, and organoleptic traits), as well as, selecting those with comparative advantages especially in fruit texture and appearance, as these are the sensory attributes with higher price premiums for marketing (Botelho et al., 2014).

This study was conducted to access the germplasm diversity of a Portuguese tomato collection, based on morphological, agronomical, chemical and molecular analysis, in order to identify the best genotypes to be used in a selection breeding program.

2. Material and methods

2.1. Plant material and samples preparation

The plant material used in this study was obtained from germplasm regeneration of 20 accessions of table tomato local varieties selected among the germplasm collection of the Portuguese Genebank (BPGV). Table 1 and Supplementary Table 1 present the BPGV accession codes, local name, type of variety, origin and place of recollection, and the corresponding Global Position System (GPS) coordinates.

Within the scope of germplasm regeneration, usually performed in genebanks, the seeds of the selected 20 accessions were cultivated on the BPGV experimental fields in Braga, Portugal, following international Genebank Standards (FAO, 2014; UPOV, 2013). For each accession, seedlings were established in flats with potting mix for one month and transplanted to the field one month after. The plantation followed a complete randomized block design with two blocks and 30 plants per accession. To reduce abiotic effects on the chemical composition of the analyzed plant material (Pinela et al., 2019), and to avoid cross pollination, the 30 plants of each accession were grown in a large box covered with net. All accessions were subjected to the same agronomic requirements.

The plantation site is characterized by a humic cambisol soil, 75–80% relative humidity, average temperatures ranging from 6.8 to 15.4 °C (mean of 9.3 °C) in April and from 14.5 to 27 °C (mean of 20.2 °C) in July, and an average rainfall of 105.4 mm in April and 7.1 mm in July. More details on the geographical and edaphoclimatic characteristics of the location are shown in Supplementary Table 3.

Agronomical and morphological measurements were performed on 10 plants of each accession population, during the crop cycle.

For molecular analysis, fresh leaves were collected from 5 to 10 plants of each accession. The leaves collected for DNA extraction and analysis were dehydrated on silica gel for 24 h and kept at –20 °C during the extraction process until the end.

The ripe fruits were used for compositional analysis. Moreover, as the main purpose was germplasm regeneration, the fruits available for analysis considered also the need of fruits for seed extraction, according to specific guidelines and criteria defined by the International Plant Genetic Resources Institute (IPGRI, 1996) and the International Union for the Protection of New Varieties of Plants, Guideline TG/44/10 for tomato (UPOV, 2013). Therefore, such fruits were progressively harvested at full maturity of seeds (*i.e.*, after the seeds reached physiological maturity), which was determined according to criteria of IPGRI (1996) descriptors for tomato. The ripe fruits of each accession were immediately transported to the laboratory where main characteristics and measurements of size and weight were recorded (Table 1) before being stored at −20 °C. Afterwards tomatoes were lyophilized (FreeZone 4.5 model 7750,031, Labconco, Kansas City, USA) and then reduced to a fine dried powder that was kept at −20 °C until analysis.

2.2. Molecular analysis

2.2.1. Molecular characterization using SSR markers

Genomic DNA was isolated from the set of dehydrated leaves harvested at each accession using an E.Z.N.A. Plant DNA Kit (Omega biotek, Norcross, GA, US) with adapted protocol (on step 4, the incubation was done at 65 °C for 20 min with four inverting tubes and, in step 12, it was at 65 °C for 3 min, then the temperature was lowered to 55 °C and 5 μL of proteinase K were added to each sample, followed by incubation at 55 °C for 90 min). The bulk DNA by accession resulted from mix obtained with equal amount of each leaf collected in the accession. Genotyping was performed using 250 ng of DNA per accession. Seven simple sequence repeat (SSR) markers were used to assess the diversity among accessions (Supplementary Table 4). These microsatellites were previously identified to assess parental polymorphisms through 224 SSR markers in the Sol Genomics Network database (Yogendra and Ramanjini Gowda, 2013). Among these *loci*, two have a core motif TG or CA and two each have GA or GC. PCRs were carried out in a final volume of 20 μL, containing 10 mM Tris-HCl, 50 mM KCl, 2 mM $MgCl_2$, 100 μM of each dNTP, 0.4 μM of each primer, 20 ng genomic DNA, and 1 Unit of *Taq* DNA polymerase. DNA amplification was performed with 35 cycles of 94 °C for 30 s, 57 °C for 30 s, 72 °C for 30 s, followed by a final 5 min extension at 72 °C. PCR products were initially fractionated by 6% SDS-PAGE and visualized by silver staining. The fingerprints of the 20 accessions were combined together for further analysis.

2.2.2. Data analysis

The genetic diversity among accessions was estimated based on the number of alleles (Na), Shannon's coefficient (I), observed and expected heterozygosity (Ho, He), unbiased expected heterozygosity (uHe), and fixation index (F) of the seven microsatellites used in the analysis. Na means all different alleles detected at an SSR *locus*, and it reflects the allelic richness in a population. The polymorphic information content (PIC) value ($PIC = 1 - \Sigma P_i^2$, here, P_i is the allele frequency of the i^{th} alleles

Table 1
List of the 20 characterized table tomato accessions, included in the BPGV Portuguese germplasm collection, whose ripe fruits were used for compositional analysis. The accessions are ordered by code number, with details about common name, commercial type, basic features of the plants and fruits of each accession, and geographic origin of the collected germplasm.

Tomato accession main features				Basic description of the ripe fruit					Geographic origin	
Accession number	PT local name (literal meaning in EN)	Similar commercial type	Plant growth type	Fruiting period (d)	Predominant fruit shape	Fruit weight (g)	Number of locules	Fruit size	Region	Collecting site
BPGV11098	Tomate maçã (apple tomato)	Flattened tomato	Indeterminate	79	Flattened	504.0	11	Very large	Lisbon	Manique do Intendente, Azambuja
BPGV11350	Tomate de cacho, liso e redondo (round and smooth fruits in vines)	Round standard	Indeterminate	85	Slighthy flattened	139.0	5	Intermediate	Lisbon	Manique do Intendente, Azambuja
BPGV11363	Tomate coração-de-boi (oxheart tomato)	Beefsteak tomato	Indeterminate	85	Heart-shaped	379.0	11	Large	Santarém	Arrouquelas, Rio Maior
BPGV11372	Tomate	Round standard	Indeterminate	86	Heart-shaped	344.0	11	Large	Leiria	Arrimal, Porto de Mós
BPGV11400	Tomate maçã (apple)	Flattened tomato	Determinate	89	Slightly flattened	154.0	5	Intermediate	Santarém	Amiais de Baixo, Santarém
BPGV11465	Tomate vermelho (red)	Round standard	Determinate	88	Slightly flattened	218.0	6	Intermediate	Santarém	Carvoeiro, Mação
BPGV11681	Tomate	Round standard	Semi-determinate	71	Slightly flattened	144.5	4	Intermediate	Santarém	Bemposta, Abrantes
BPGV11696	Tomate redondo (round tomato)	Round standard	Semi-determinate	77	Slightly flattened	123.5	6	Intermediate	Santarém	São José da Lamarosa, Coruche
BPGV11732*	Tomate coração-de-boi (oxheart tomato)	Beefsteak tomato	Indeterminate	77	Slightly flattened	196.1	6	Large	Santarém	Chouto, Chamusca
BPGV11803	Tomate grosso (thick fruit tomato)	Round standard	Semi-determinate	71	Slightly flattened	214.0	10	Large	Portalegre	Santa Maria de Marvão
BPGV11907	Tomate cabecinhas (small heads tomato)	Plum tomato	Determinate	62	Cylindrical	92.5	3	Small	Portalegre	Aldeia Velha, Avis
BPGV12260	Tomate coração-de-boi (oxheart tomato)	Beefsteak tomato	Determinate	81	Slightly flattened	168.0	6	Intermediate	Bragança	Santulhão, Vimioso
BPGV12437	Tomate amarelo (yellow tomato)	Yellow tomato	Determinate	65	Slightly flattened	190.8	6	Intermediate	Bragança	Águas Vivas, Miranda do Douro
BPGV12446*	Tomate antigo (old tomato)	Beefsteak tomato	Indeterminate	65	Flattened	407.5	10	Large	Bragança	Águas Vivas, Miranda do Douro
BPGV12465	Tomate sem varas (no staking tomato)	Round standard	Determinate	65	Rounded	123.0	5	Intermediate	Bragança	Peredo da Bemposta, Mogadouro
BPGV12506	Tomate coração-de-boi (oxheart tomato)	Beefsteak tomato	Semi-determinate	83	Heart-shaped	260.0	11	Large	Santarém	Santarém
BPGV12906	Tomate	Elongated Roma	Indeterminate	80	Cylindrical	189.0	4	Intermediate	Aveiro	Frossos, Albergaria-a-Velha
BPGV12954	Tomate coração-de-boi (oxheart tomato)	Beefsteak tomato	Indeterminate	88	Heart-shaped	246.0	10	Intermediate	Aveiro	Válega, Ovar
BPGV13034	Tomate coração-de-boi (oxheart tomato)	Beefsteak tomato	Indeterminate	85	Heart-shaped	415.0	12	Large	Guarda	Teixeira, Seia
BPGV16388	Tomate salada (salad tomato)	Roma tomato	Determinate	85	Cylindrical	126.3	2	Intermediate	Castelo Branco	Proença-a-Nova

Note: Basic description of the ripe fruits was assessed in 10 plants from the population of each accession. Data correspond to the mean values for each accession (see Supplementary Table 2). Fruit size: very large >10 cm; large (8.1–10 cm); intermediate (5.1–8 cm); small (3–5 cm). *Accessions BPGV11732 and BPGV12446 did not produce enough fruits to fulfil regeneration purpose and compositional analysis therefore, the fruits of these two were not analyzed.

of the *locus*) takes into account both the allelic richness and the relative frequency of each allele, and is a very effective index in diversity evaluation. The genetic distances among populations were calculated using GenAlex6.53 (Peakall and Smouse, 2012) in which the calculation principle was referred to Nei (1973). A principal coordinate analysis (PcoA), assayed on populations based on SSR data, was performed on the bootstrap by genetic distance matrix using the GenAlex 6.5 software (Peakall and Smouse, 2012).

2.3. Agronomical and morphological characterization

Thirty-six morphological and agronomical descriptors were used, namely 3 plant descriptors, 6 leaf descriptors, 6 flowers/inflorescence descriptors, and 21 fruit descriptors (Supplementary Table 3), according to descriptors and guidelines proposed by IPGRI (1996) and UPOV (2013). Phenological data were recorded during flowering, fruiting, and harvest. Attitude, type and size of leaves, plant size, and growth type were registered for leaf and plant morphology. Inflorescence type and attitude, number of inflorescences, number of flowers per inflorescence, fruit shape and size, matured fruit color, pedicel length, and number of locules were recorded for flower and fruit morphology. Fruit weight and plant yield were measured for agronomic performance. Flower and fruit colors were determined using the color chart scales of the Royal Horticultural Society (RHS, 2015).

2.4. Compositional analysis

2.4.1. Proximate composition

The tomato samples were analyzed for moisture, protein, fat, and ash contents following AOAC procedures (AOAC International, 2016). Briefly, the protein content ($N \times 6.25$) was estimated by the macro-Kjeldahl method, using an automatic distillation and titration unit (Pro-Nitro-A, Selecta, Spain); the crude fat content was determined by Soxhlet extraction with petroleum ether; and the ash content was determined by incineration in a muffle furnace at 550 ± ± 15 °C. Total carbohydrates were calculated by difference: 100 – (g moisture + g protein + g fat + g ash). The results were expressed as g per 100 g of fresh weight. The energy value was calculated according to the Regulation (EU) No 1169/2011 (EU, 2011) as follows: 4 × (g protein + g carbohydrates) + 9 × (g fat) and given as kcal per 100 g of fresh weight.

2.4.2. Hydrophilic compounds

Free sugars were determined by high performance liquid chromatography (HPLC) coupled to a refraction index (RI) detector as previously described by Barros et al. (2013). The mobile phase consisted of acetonitrile/deionized water (70:30, *v/v*, acetonitrile HPLC-grade, Lab-Scan, Lisbon, Portugal) and separation was achieved using a Eurospher 100-5 NH2 column (4.6 × 250 mm, 5 μm, Knauer). The compounds were identified by chromatographic comparisons with authentic standards and quantified by the internal standard (IS) method (IS, melezitose, Sigma-Aldrich, St. Louis, MO, USA). The results were recorded and processed using Clarity 2.4 software (DataApex, Prague, Czech Republic) and expressed as g per 100 g of fresh weight.

Organic acids were analyzed by ultra-fast liquid chromatography (Shimadzu 20 A series UFLC, Shimadzu Corporation, Kyoto, Japan) coupled to a photodiode array detector (PDA), operating in the conditions previously described by Pereira et al. (2013). The chromatographic separation was achieved with a SphereClone reverse phase C18 column (250 × 4.6 mm, 5 μm, Phenomenex, Torrance, CA, USA). The compounds were identified and quantified by comparing the area of the sample' peaks recorded at 215 nm or 245 nm (for ascorbic acid) with calibration curves obtained from commercial standards (Sigma-Aldrich, St. Louis, MO, USA). The results were recorded and processed using LabSolutions Multi LC-PDA software (Shimadzu Corporation, Kyoto, Japan) and expressed as mg per 100 g of fresh weight.

2.4.3. Lipophilic compounds

Fatty acids were analyzed by gas chromatography (DANI GC 1000, Contone, Switzerland) equipped with a split/splitless injector and a flame ionization detector (GC-FID at 260 °C) operating in the conditions previously described by Barros et al. (2013). Separation was achieved with a Macherey-Nagel column (30 $m \times 0.32$ mm ID $\times$ 0.25 μm d_f). The fatty acids were identified and quantified by comparing the relative retention times of the FAME (fatty acid methyl esters reference standard mixture 37, Sigma-Aldrich, St. Louis, MO, USA) standard with the ones of the sample' compounds. The results were recorded and processed using CSW 1.7 software (Data Apex 1.7, Prague, Czech Republic) and expressed as relative percentage of each fatty acid.

Tocopherols were determined following a procedure previously described by Barros et al. (2013), using a HPLC system (Knauer, Smartline system 1000, Berlin, Germany) coupled to a fluorescence detector (FP-2020; Jasco, Easton, USA) programmed for excitation at 290 nm and emission at 330 nm, using the IS (tocol, Matreya, Pleasant Gap, PA, USA) method for quantification. The mobile phase consisted of hexane/ethyl acetate (70:30, *v/v*, hexane and ethyl acetate HPLC-grade, Lab-Scan, Lisbon, Portugal), and chromatographic separation was performed using a Polyamide II column (250 × 4.6 mm, 5 μm, YMC, Kyoto, Japan). The results were recorded and processed using Clarity 2.4 software (DataApex, Prague, Czech Republic) and expressed as μg per 100 g of fresh weight.

Carotenoids and *chlorophylls* were simultaneously determined by a method previously optimized by Nagata and Yamashita (1992), which is based on the extraction with acetone/hexane (4:6) and measurement of the optical density of the supernatant at 453, 505, 645, and 663 nm. The contents were calculated according to the following equations and expressed as mg per 100 g of fresh weight.

$$\beta - Carotene = 0.216 \times A_{663} - 1.220 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453} \quad (1)$$

$$Lycopene = -0.0458 \times A_{663} + 0.204 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453} \quad (2)$$

2.4.4. Statistical analysis

Data results were expressed as mean ± standard deviation. All statistical tests were performed at a 5% significance level using SPSS Statistics software (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Differences between blocks (accessions and groups of accessions) were analyzed using one-way analysis of variance (ANOVA). The fulfilment of the ANOVA requirements, specifically the normal distribution of the residuals and the homogeneity of variance, was tested by means of the Shapiro Wilk's and the Levene's tests, respectively. All dependent variables were compared using Tukey's honestly significant difference (HSD) or Tamhane's T2 multiple comparison tests, when homoscedasticity was verified or not, respectively. In addition, a linear discriminant analysis (LDA) was performed to compare the different tomato accessions, considering all compositional data, according to each genotype, the fruit size and shape, their geographic origin, the individual values of observed heterozygosity, and the genetic distance between populations. The stepwise technique and the Wilk's λ test with an F-value of 3.84 for entering and 2.71 for removal of variables were applied.

3. Results and discussion

3.1. Molecular characteristics

3.1.1. Genetic diversity among tomato genotypes

The 20 accessions characterized in this study represent most of the diversity of the tomato collection of the Portuguese Genebank (BPGV). To use, maintain, and increase efficiently this crop germplasm collection, it is important to assess the inherent genetic diversity. Morphological descriptors do not always allow the quantification of genotypic differences, since the quantitative characters can be altered by environmental factors (Cooke et al., 1995). In contrast, molecular markers can provide an effective tool for variety identification as they are independent of environmental effects (Lee and Henry, 2001; Sim et al., 2009). Among the different available marker systems, SSR or microsatellite markers have been widely used in genetic diversity analysis of crops such as tomatoes (He et al., 2003; Pawar et al., 2016; Yogendra and Ramanjini Gowda, 2013) and were selected for this study because of their high reproducibility, multiallelic traits, codominant inheritance, relative abundance, and wide genome coverage (Varshney et al., 2005). The selected SSR markers are related to fruit shelf-life, fruit firmness, total soluble solids, and lycopene (Supplementary Table 4).

A total of 19 different alleles were detected at the 7 SSR *loci* among the 20 Portuguese tomato accessions described in Table 1, which captured 77.5% of the total alleles, suggesting that these markers constitute reliable tools for the exploitation of the genetic variability present in the tomato germplasm resource in Portugal.

The allele frequencies of the different microsatellite markers are given in Table 2. The number of alleles (NA) varied among *loci*, from six alleles at LEga007 (214, 216, 218, 222, 224, and 228) to only one allele at LEga005 (271), with an average of three alleles per *locus*. The distribution frequencies of the alleles ranged from 0.05 in LEga005 to 0.32 in LEga007. Among the 19 alleles, 5% were exclusive, meaning that they were unique to only one tomato accession (allele 254 in BPGV16388). Some alleles were comparatively less prevalent including the allele 167 (0.05) of SSR310 in BPGV11907, allele 212 (0.05) of LEaat003 in BPGV11400 and BPGV12437, allele 224 (0.05) of LEg007 in BPGV12437 and BPGV13034, and allele 216 (0.075) of LEg007 in BPGV12506 and BPGV16388.

The mean observed heterozygosity (Ho = 0.34) was similar to the mean expected heterozygosity (He = 0.38). The *locus*-wise heterozygosity statistics revealed maximum heterozygosity for Leg007 and LEaat006 (Ho = 1.00) and minimum for LEaa7 (Ho = 0.05) markers. The fixation index (F) ranged from −0.572 (LEaat006) to 1.000 (SSR310) with an average $F = 0.23$ (Table 2). The Shannon's information index (I) for a particular *locus* was directly proportional to the number of alleles ($R^2 = 0.86$). The accession-wise Ho ranged from 0.421 to 0.579 with an average of 0.47 (Fig. 1A). All these data provided important information on the selection of SSR *loci* in diversity analysis for the Portuguese tomato genotypes.

3.1.2. Genetic distance among tomato accessions

The cluster analysis with the first two principal components produced by PcoA analysis suggested that there were obvious differentiations among the 20 accessions (Fig. 1B). Coordinate 1 provided a clear separation of the markers into two main groups. These groups may somehow be related to morphological, agronomic or compositional characteristics; for example, in the top-left quartile, BPGV12906 and BPGV16388 were both in cylindrical shape, and BPGV12954, BPGV11372, and BPGV11363 exhibit a heart-shaped profile. The two accessions (BPGV11732 and BPGV12446) that did not produce enough fruits to fulfil regeneration purpose and compositional analysis were also found in the same quartile of the biplot. The genetic differentiation among tomato genotypes thus provided useful information for parental selection in breeding and other genetic studies. In future work, it would be important to exploit an increased number of both markers and accessions to broaden the genetic base in tomato breeding programs.

Table 2
Genetic information resulting from the characterization of the 20 Portuguese tomato accessions from the BPGV collection. Part A: allele frequency of the seven SSR markers. Part B: number of alleles (Na), number of effective alleles (Ne), Shannon'e coefficient (I), observed and expected heterozygosity (Ho, He), unbiased expected heterozygosity (uHe), and fixation index (F) of the 7 microsatellites and 20 accessions.

	LEaat006	LEaat003	LEaat007	LEga005	LEga007	SSR310	SSR45	Mean ± SD
Part A: Allele frequency								
117			0.525					
120			0.475					
158	0.250							
160	0.475							
167						0.050		
170	0.275							
170						0.950		
212		0.050						
214					0.250			
216					0.075			
218					0.175			
221		0.950						
222					0.225			
224					0.050			
228					0.225			
254							0.025	
269							0.575	
271				1.000				
272							0.400	
Total	0.16	0.11	0.11	0.05	0.32	0.11	0.16	
Part B: Microsatellites information								
Na	3	2	2	1	6	2	3	2.71±0.61
Ne	2.749	1.105	1.995	1.000	4.938	1.105	2.036	2.13±0.53
I	1.055	0.199	0.692	0.000	1.667	0.199	0.777	0.66±0.22
Ho	1.000	0.100	0.050	0.000	1.000	0.000	0.200	0.34±0.17
He	0.636	0.095	0.499	0.000	0.798	0.095	0.509	0.38±0.12
uHe	0.653	0.097	0.512	0.000	0.818	0.097	0.522	0.39±0.12
F	-0.572	-0.053	0.900	n/a	-0.254	1.000	0.607	0.27±0.25

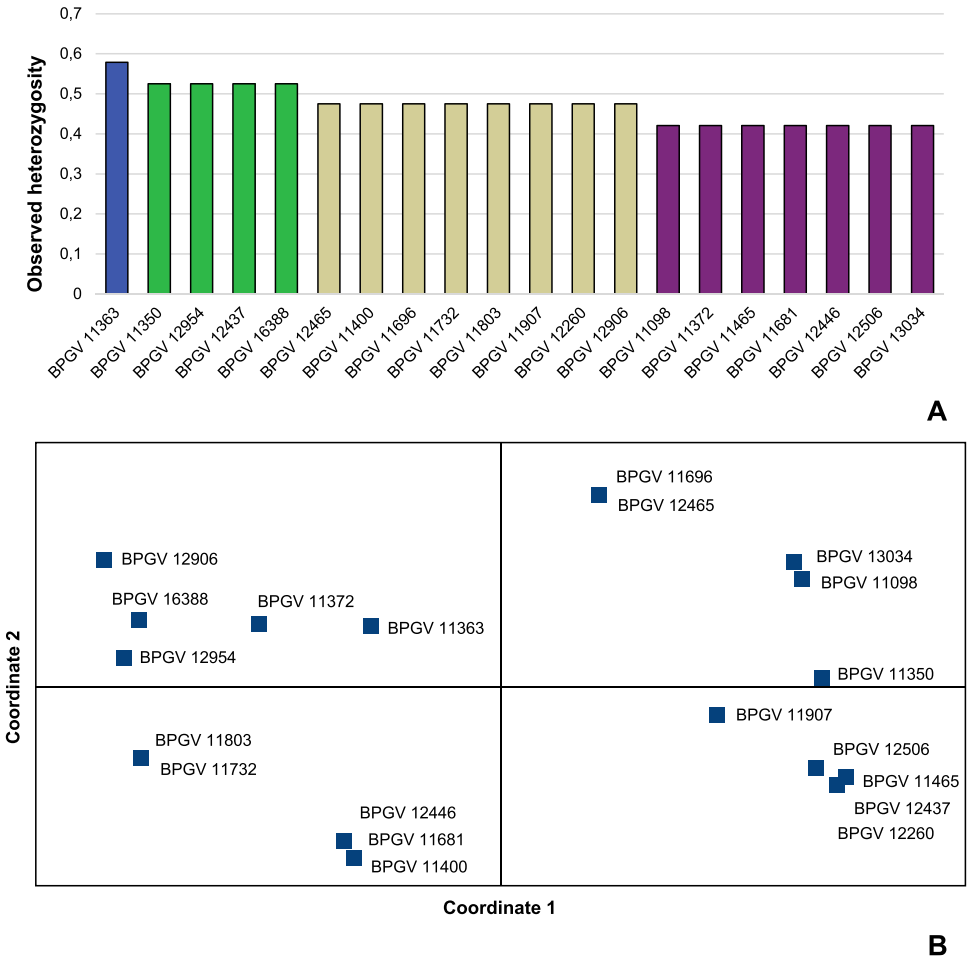

Fig. 1. Individual values of observed heterozygosity for the 7 microsatellites in the 20 Portuguese tomato genotypes presented in Table 1 (A) and biplot of the principal coordinate analysis (PCoA) carried out with the same genotypes evaluated with seven SSR markers (B).

3.2. Agronomic features and morphological traits

The PCoA classified the 20 table tomato accessions into four groups of five accessions each (Fig. 1B). Thus, the 20 accessions were clustered according to these groups in Table 3 and Supplementary Table 5, where their morphological and agronomic characteristics are described, considering the descriptors and guidelines proposed by IPGRI (1996) and UPOV (2013), which are also summarized in Supplementary Table 3. An overview of the selected main traits showed that:

- The most frequently displayed grow habit was the indeterminate type (45% of the accessions), while 35% of the accessions had a determine growth habit and 20% semi-determinate.
- Standard (50% of the accessions) was the predominant leaf type, followed by Potato (25%) and Peruvianum (25%) leaf types. Considering the leaf blade, 85% of the accessions had pinnate and 15% bipinnate leaves.
- The main inflorescence type corresponded to the generally uniparous trait (60% of the accessions); however, other types such as both uniparous and multiparous (25%) and generally multiparous (15%) types were also present.
- The number of inflorescences, which was evaluated on the main stem, did not seem to be related to the growth habit of the accessions. Both higher and lower values for this descriptor (number of inflorescences) were observed in either indeterminated and determinated habits, as well as in small, intermediate, and large plant sizes. The accessions BPGV12260 and BPGV16388 had the highest number of inflorescences per plant (6 inflorescences each). The lowest number of inflorescences observed was 3 per plant (*e.g.*, BPGV11372, BPGV11681, BPGV11803, and BPGV12446). When comparing the four clusters of accessions, significant differences ($p < 0.05$) are observed for this descriptor mainly among samples in the bottom-left (less inflorescences, ~3.6) and bottom-right (more inflorescences, 5.2) quartiles of the PCoA biplot (Fig. 1B).
- The descriptor number of flowers per inflorescence (measured in the second inflorescence of the main stem) showed great variation between accessions, ranging from 3 to 20 flowers per inflorescence. BPGV11803 registered the highest number of flowers per inflorescence (20) and BPGV11098 had the lower number (3). Considering these results, the greater number of flowers set did not seem to be related to the greater number of inflorescences formed, as observed in BPGV11803 (*e.g.*, average of 3 inflorescences per plant and 20 flowers per inflorescence). However, the inflorescence type such as the generally multiparous type may be correlated with a higher number of flowers per inflorescence.
- The trait corolla color did not vary; all accessions had yellow corolla flowers.
- The fruits revealed some variations in their characteristics between accessions. For instance, tomatoes exhibited dissimilarities considering the descriptors fruit shape, the ribbing at the calyx ending, the number of locules per fruit.
- The fruiting period varied between more or less two and three months after planting. BPGV11907 had the shortest period (62 days) and BPGV11400 the longest one (89 days).

Table 3
Main morphological traits of the 20 BPGV table tomato accessions clustered according the PCoA. Descriptors according toIPGRI (1996) and UPOV (2013).

Accession number	Leaves		Flowers/Inflorescence			Fruits							
	Leaf type	Type of leaf blade	Inflorescence type	Number of inflorescences	Number of flowers per inflorescence	Exterior color of mature fruit	Fruit size homogeneity	Fruit length (mm)	Fruit width (mm)	Ribbing at calyx end	Fruit shoulder shape	Fruit cross-sectional shape	Fruit blossom end shape
BPGV11363	Peruvianum	Bipinnate	Generally multiparous	5	11	Orange-Red Group	Intermediate	91.4	95	Weak	Slightly depressed	Angular	Flat to pointed
BPGV11372	Potato leaf type	Bipinnate	Both	3	8	Red Group	Intermediate	109	90.1	Very strong	Strongly depressed	Irregular	Pointed
BPGV12906	Potato leaf type	Pinnate	Both	4	8	Orange-Red Group	Intermediate	119	57.1	Intermediate	Slightly depressed	Angular	Flat to pointed
BPGV12954	Standard	Pinnate	Both	4	12	Orange-Red Group	Intermediate	79.7	76.9	Intermediate	Slightly depressed	Round	Flat to pointed
BPGV16388	Standard	Pinnate	Both	6	8	Red Group	Low	65.1	63.6	Weak	Slightly depressed	Irregular	Flat
Average				**4±1** ^{A,B}	**9±2** ^{A}			**93±19** ^{A}	**77±15** ^{A}				
BPGV11098	Standard	Pinnate	Generally uniparous	5	3	Red Group	Low	85.3	111	Strong	Strongly depressed	Irregular	Indented to flat
BPGV11350	Standard	Pinnate	Generally uniparous	5	11	Red Group	Low	58.9	63.1	Weak	Slightly depressed	Round	Flat
BPGV11696	Standard	Pinnate	Generally uniparous	5	5	Red Group	Low	54.7	63.9	Very weak	Slightly depressed	Round	Flat
BPGV12465	Potato leaf type	Pinnate	Generally uniparous	4	7	Red Group	Low	61.9	60.7	Weak	Slightly depressed	Round	Flat
BPGV13034	Peruvianum	Pinnate	Generally multiparous	5	10	Red Group	Intermediate	101.5	99	Intermediate	Moderately depressed	Irregular	Flat to pointed
Average				**4.8±0.4** ^{A,B}	**7±3** ^{A}			**72±18** ^{A}	**80±21** ^{A}				
BPGV11465	Potato leaf type	Pinnate	Generally uniparous	5	6	Red Group	Low	60.9	79.9	Weak	Slightly depressed	Round	Indented to flat
BPGV11907	Standard	Pinnate	Generally uniparous	5	6	Red Group	Low	72.6	50.3	Very weak	Flattened	Round	Flat to pointed
BPGV12260	Peruvianum	Pinnate	Generally uniparous	6	6	Red Group	Low	64.1	68.5	Weak	Slightly depressed	Irregular	Flat
BPGV12437	Standard	Pinnate	Generally uniparous	5	5	Orange Group	Low	58.8	75.8	Weak	Moderately depressed	Angular	Indented to flat
BPGV12506	Peruvianum	Pinnate	Generally uniparous	5	8	Red Group	Intermediate	89.6	89.6	Intermediate	Slightly depressed	Angular	Flat to pointed
Average				**5.2±0.4**^{A}	**6±1** ^{A}			**69±11** ^{A}	**73±13** ^{A}				
BPGV11400	Standard	Bipinnate	Generally uniparous	5	6	Red Group	Low	58.7	63.8	Weak	Slightly depressed	Round	Flat
BPGV11681	Standard	Pinnate	Generally uniparous	3	4	Red Group	Low	66.9	62.4	Intermediate	Slightly depressed	Angular	Flat to pointed
BPGV11732	Standard	Pinnate	Generally uniparous	4	6	Red Group	Low	74.2	84.3	Intermediate	Slightly depressed	Irregular	Flat
BPGV11803	Potato leaf type	Pinnate	Generally multiparous	3	20	Red Group	Low	63.7	82.4	Weak	Slightly depressed	Irregular	Flat
BPGV12446	Peruvianum	Pinnate	Both	3	9	Red Group	Low	68.4	109	Very strong	Strongly depressed	Irregular	Indented to flat
Average				**3.6±0.8** ^{B}	**9±6** ^{A}			**66±5** ^{A}	**78±17** ^{A}				

For more information about descriptors and scores for morphological characterization see Supplementary Table 3. In each column, different capital letters indicate that at least one of the four groups of tomato accessions established by the PCoA differs statistically ($p < 0.05$) from the others.

Particular attention was given to fruit morphologic and agronomic features (Tables 1 and 3). All observations were taken, when possible, on the 3rd fruit of the 2nd and/or 3rd truss at the full maturity stage, average of 10 fruits from different plants (UPOV, 2013). Thus, some of the mature fruit relevant traits are described below:

- The fruits exhibited five different shapes: 1 accession (BPGV12465) corresponded to a rounded shape fruit; 2 accessions (BPGV11098 and BPGV12446) had flattened fruits; 3 accessions (BPGV11907, BPGV12906 and BPGV16388) matched cylindrical shape; 5 accessions (BPGV11363, BPGV11372, BPGV12506, BPGV12954 and BPGV13034) exhibited a heart-shaped profile; and the remaining 9 accessions were related to a slightly flattened shape.
- The exterior fruit color was evaluated using the RHS color chart scales (RHS, 2015) at the full maturity phase. Red was the predominant color in 80% of the accessions. Only 3 accessions (BPGV12906, BPGV12954 and BPGV11363) were orange-red and 1 accession (BPGV12437) was orange.
- The descriptor fruit size homogeneity categorized the accessions into two classes, with 70% of the accessions belonging to the low class and 30% to the intermediate class.
- The trait ribbing at calyx end showed great variability. It was absent or very weak in BPGV11696 and BPGV11907, strong in BPGV11098, and very strong in BPGV11372 and BPGV12446. Considering the remaining accessions, 45% present weak ribs and 30% intermediate ribs at the end of the calyx.
- The fruit cross-sectional shape matched the categories irregular (40% of the accessions), round (35%), and angular (25%).
- Regarding the fruit blossom end shape descriptor, the accessions were distributed into four categories: flat (40%), flat to pointed (35%), indented to flat (20%), and pointed (5% of the accessions).
- The feature fruit shoulder shape observed correspond to the following categories: 70% of the accession had fruits shoulder Slightly depressed, 15% strongly depressed, 10% Moderately depressed, and 5% were Flattened.
- The longest fruit length, recorded from stem end to blossom end, to one decimal place, at maturity, were observed within BPGV12906 (119 mm), BPGV11372 (109 mm), and BPGV13034 (101.5 mm). The smallest recorded fruit lengths refer to BPGV11696 (54.7 mm), BPGV11400 (58.7 mm) and BPGV12437 (58.8 mm).
- On the other hand, considering the trait fruit width, the highest values corresponded to the accessions BPGV11098 (111 mm) and BPGV12446 (109 mm). The lowest value was 50.3 mm (accession BPGV11807).
- Three accessions produced very large fruits (size at maturity >10 cm) with the highest recorded weights: BPGV11098 (504 g), BPGV13034 (415 g), and BPGV12446 (407.5 g). Fruits of the accession BPGV11907 were small (3–5 cm) and had the smallest fruit weight (92.5 g).
- The number of locules (spaces derived from carpels merging, containing seeds) is a very important trait influencing the fruit shape and size. The number of locules varied from two (BPGV16388) up to (accession BPGV13034).

Overall, the characters showing higher variability were related with the number of flowers per inflorescence and fruit morphology, such as fruit weight, fruit shape, the ribbing at the calyx ending, and number of locules per fruit. This diversity was somehow expected considering de popular/local names attributed to these tomato farmer's varieties, which reflect some agromorphological and organoleptic features of the plants and their fruits. However, some accessions with similar names, such as those so-called oxheart tomato, showed recognizable differences in several of the reported descriptors.

3.3. Nutritional characteristics

The results of the chemical analysis concern the fruits of 18 of the 20 tomato accessions, since BPGV11732 and BPGV12446 did not produce enough fruits to fit regeneration purposes and compositional analysis. In this section, to access whether compositional variations may be related to the genetic distance that characterizes the tomato germplasm collection, the composition tables were organized with the accessions clustered according to the PCoA biplot quartiles (Fig. 1B).

3.3.1. Proximate composition

The results of the proximate composition and energy values of the 18 tomato accessions and the 4 clusters are presented in Table 4. All samples had moisture contents $\geq$ 93.9 g 100 g^{-1}. Carbohydrates were abundant macronutrients and varied from 3.49 to 5.42 g 100 g^{-1} in BPGV13034 and BPGV11681, respectively. BPGV13034 also revealed a low energy value (17.49 kcal 100 g^{-1}), although it was the accession with the highest protein content (0.75 g 100 g^{-1}). In general, all tomato accessions were characterized by low protein (0.10–0.75 g 100 g^{-1}) and fat (0.04–0.09 g 100 g^{-1}) contents, which makes them suitable foods for low-calorie diets. The ash (mineral) content was particularly high in BPGV11465 and BPGV16388, with 0.61 and 0.65 g 100 g^{-1}, respectively. Comparable values of moisture (94.5 g 100 g^{-1}) and ashes (0.50 g 100 g^{-1}) and slightly higher of protein (0.88 g 100 g^{-1}) and fat (0.20 g 100 g^{-1}) are reported in the United States Department of Agriculture (USDA) Food Composition Database for ripe red tomato (USDA 2016). Guil-Guerrero e Rebolloso-Fuentes (2009) reported a similar moisture content (~93–96 g 100 g^{-1}), but slightly higher protein (0.55–1.05 g 100 g^{-1}), fat (0.20–0.67 g 100 g^{-1}), and ash (0.75–1.41 g 100 g^{-1}) levels in eight tomato varieties from Almería, Spain. Another study described a higher carbohydrate content (5.14–7.99 g 100 g^{-1}) and energy value (23.72–34.67 kcal 100 g^{-1}) in four tomato farmers' varieties in north-eastern Portugal homegardens (including the oxheart and round varieties) (Pinela et al., 2012).

When comparing the four clusters established by the PCoA, it can be seen that they only differed in the carbohydrates content (Table 4). Thus, despite its genetic distance, most of its proximate constituents did not differ ($p < 0.05$).

3.3.2. Composition in free sugars and organic acids

Table 5 shows the free sugar composition of the tomato accessions described in Table 1, and the mean values of each cluster defined based on the PCoA biplot (Fig. 1B). Fructose was the most abundant sugar followed by glucose and then by sucrose, which was not detected in all samples. The highest fructose contents (~2.5 g 100 g^{-1}) were detected in BPGV11681, BPGV11907 and BPGV11372, which, together with BPGV11400, BPGV12906, and BPGV12260, also had the highest glucose and total sugar contents (reaching 1.83 and 4.30 g 100 g^{-1} in BPGV11681, respectively). Interestingly, the three tomato genotypes known only as "tomato" (Table 1) were characterized by having high sugar contents. On the other hand, BPGV11465 and BPGV12506 (red and oxheart tomatoes, respectively), both originating in Santarém, were characterized by low fructose, glucose and total sugar contents and did not present sucrose. Higher levels of fructose (2.71 g 100 g^{-1}), glucose (2.22 g 100 g^{-1}) and total sugars (4.95 g 100 g^{-1}) were already reported by Pinela et al. (2012) for oxheart tomato from Miranda do Douro (north-eastern Portugal). The total sugar levels found in the studied tomato genotypes comprise the value given by the USDA database (2.63 g 100 g^{-1}) for ripe red tomatoes (USDA 2016).

As verified in Table 4 for carbohydrates, the free sugar content also varied among the four clusters of tomato accessions (Table 5). Samples from the left quartiles of the PCoA biplot (Fig. 1B) seemed to be closer in terms of carbohydrates and free sugar content, while the main differences were found between those from the bottom-left (*e.g.*, BPGV11400) and top-right (*e.g.*, BPGV11098) quartiles, so with a greater genetic distance.

Table 4
Proximate composition and energy of the ripen fruits of the BPGV tomato accessions clustered according the PCoA.

Accession	Moisture (g 100 g^{-1})	Proteins (g 100 g^{-1})	Fat (g 100 g^{-1})	Ash (g 100 g^{-1})	Carbohydrates (g 100 g^{-1})	Energy (kcal 100 g^{-1})
BPGV11363	94.8±0.4 ^{f,g}	0.096±0.002 ^{k}	0.050±0.001 ^{f,g}	0.453±0.001 ^{f,g}	4.60±0.01 ^{e}	19.21±0.02 ^{h}
BPGV11372	94.4±0.1 ^{h}	0.120±0.002 ^{i}	0.057±0.001 ^{c,d,e,f}	0.49±0.01 ^{c,d,e}	4.91±0.01 ^{b}	20.63±0.04 ^{f}
BPGV12906	94.7±0.3 ^{g}	0.67±0.01 ^{c}	0.062±0.001 ^{b,c,d,e}	0.412±0.002 ^{i,j}	4.17±0.01 ^{i}	19.93±0.01 ^{g}
BPGV12954	95.4±0.6 ^{b}	0.100±0.002 ^{j,k}	0.053±0.004 ^{e,f,g}	0.36±0.03 ^{k}	4.10±0.02 ^{j}	17.28±0.09 ^{m}
BPGV16388	94.3±0.8 ^{i}	0.149±0.002 ^{e,f,g}	0.091±0.002 ^{a}	0.650±0.005 ^{a}	4.85±0.01 ^{d}	20.82±0.03 ^{e}
Average	**94.7±0.4** ^{A}	**0.2±0.2** ^{A}	**0.06±0.02** ^{A}	**0.5±0.1** ^{A}	**4.5±0.3** ^{A,B}	**20±1** ^{A}
BPGV11098	95.3±0.3 ^{b,c}	0.100±0.0004 ^{j,k}	0.053±0.001 ^{d,e,f,g}	0.480±0.003 ^{c,d,e,f}	4.08±0.01 ^{j}	17.22±0.04 ^{m}
BPGV11350	94.4±0.7 ^{h,i}	0.16±0.01 ^{d,e}	0.050±0.001 ^{d,e,f,g}	0.47±0.02 ^{d,e,f}	4.92±0.01 ^{c}	20.78±0.05 ^{e}
BPGV11696	94.4±0.2 ^{h}	0.140±0.001 ^{g,h}	0.0600±0.0004 ^{b,c,d}	0.430±0.004 ^{g,h,i}	4.94±0.01 ^{c}	20.87±0.02 ^{d,e}
BPGV12465	94.8±0.4 ^{f}	0.133±0.003 ^{g,h,i}	0.09±0.01 ^{a}	0.48±0.01 ^{c,d,e,f}	4.46±0.01 ^{f}	19.16±0.04 ^{h}
BPGV13034	95.2±0.9 ^{c,d}	0.75±0.01 ^{a}	0.060±0.001 ^{b,c,d,e}	0.492±0.001 ^{c,d}	3.49±0.01 ^{m}	17.492±0.005 ^{l}
Average	**94.8±0.4** ^{A}	**0.3±0.3** ^{A}	**0.06±0.01** ^{A}	**0.47±0.02** ^{A}	**4.4±0.6** ^{B}	**19±2** ^{A}
BPGV11465	94.8±0.3 ^{f,g}	0.153±0.006 ^{d,e,f}	0.080±0.001 ^{a}	0.610±0.004 ^{b}	4.36±0.01 ^{g}	18.78±0.02 ^{i}
BPGV11907	94.3±0.3 ^{i}	0.143±0.002 ^{f,g,h}	0.080±0.002 ^{a}	0.420±0.001 ^{h,i,j}	5.09±0.01 ^{b}	21.65±0.01 ^{b}
BPGV12260	94.4±0.4 ^{h,i}	0.732±0.002 ^{b}	0.061±0.001 ^{b,c,d,e}	0.46±0.01 ^{e,f,g}	4.37±0.01 ^{g}	20.99±0.04 ^{d}
BPGV12437	95.5±0.7 ^{e}	0.737±0.002 ^{a,b}	0.043±0.002 ^{g}	0.469±0.004 ^{d,e,f}	3.71±0.01 ^{l}	18.18±0.02 ^{j}
BPGV12506	95.6±0.5 ^{a}	0.130±0.004 ^{h,i}	0.063±0.002 ^{b,c}	0.39±0.01 ^{j}	3.81±0.01 ^{k}	16.31±0.02 ^{n}
Average	**94.8±0.5** ^{A}	**0.4±0.3** ^{A}	**0.07±0.02** ^{A}	**0.47±0.08** ^{A}	**4.3±0.5** ^{B}	**19±2** ^{A}
BPGV11400	94.3±0.3 ^{h,i}	0.117±0.005 ^{i,j}	0.0600±0.0004 ^{c,d,e,f}	0.41±0.03 ^{i,j}	5.10±0.03 ^{b}	21.41±0.08 ^{c}
BPGV11681	93.9±0.8 ^{j}	0.169±0.003 ^{d}	0.070±0.002 ^{b}	0.45±0.03 ^{f,g,h}	5.42±0.02 ^{a}	22.98±0.07 ^{a}
BPGV11803	95.1±0.2 ^{d,e}	0.1200±0.0003 ^{i,j}	0.060±0.001 ^{c,d,e}	0.50±0.01 ^{c}	4.23±0.01 ^{h}	17.93±0.03 ^{k}
Average	**94.4±0.5** ^{A}	**0.13±0.03** ^{A}	**0.063±0.006** ^{A}	**0.45±0.04** ^{A}	**4.5±0.5** ^{A}	**21±2** ^{A}

In each column, different lowercase letters indicate that at least one tomato accession differs statistically ($p < 0.05$) from the others, while different capital letters indicate that at least one of the four groups of tomato accessions established by the PCoA differs statistically ($p < 0.05$) from the others. nd: not detected; tr: traces.

Table 5
Composition in free sugars and organic acids of the ripen fruits of the BPGV tomato accessions clustered according the PCoA.

Accession	Free sugars (g 100 g^{-1})				Organic acids (mg 100 g^{-1})				
	Fructose	Glucose	Sucrose	Total	Oxalic acid	Malic acid	Ascorbic acid	Citric acid	Total
BPGV11363	2.360±0.003 ^{c}	1.42±0.01 ^{d,e}	0.0100±0.0004 ^{b}	3.79±0.01 ^{c}	24±1 ^{g,h}	86±2 ^{d}	22.6±0.3 ^{c}	461±4 ^{c}	594±6 ^{b}
BPGV11372	2.53±0.01 ^{a}	1.580±0.004 ^{b,c}	0.0100±0.0004 ^{b,c}	4.11±0.02 ^{b}	35.1±0.4 ^{d}	186±1 ^{b}	7.4±0.1 ^{n}	254±4 ^{j}	482±5 ^{d,e}
BPGV12906	2.36±0.06 ^{c}	1.66±0.02 ^{b}	0.003±0.002 ^{c,d,e}	4.02±0.08 ^{b}	16.5±0.5 ^{m,n}	24.5±0.6 ^{l}	9.910±0.004 ^{m}	315±6 ^{g}	366±6 ^{h}
BPGV12954	2.09±0.01 ^{e,f}	1.16±0.01 ^{g,h}	nd	3.25±0.01 ^{e,f}	24.6±0.3 ^{f,g}	53.9±0.1 ^{g}	18.3±0.1 ^{e}	395±5 ^{e}	491±5 ^{d}
BPGV16388	2.20±0.05 ^{d}	1.23±0.08 ^{g,h}	0.010±0.001 ^{b}	3.44±0.02 ^{d}	8.55±0.01 ^{p}	tr	6.49±0.03 ^{o}	58±1 ^{n}	73±1 ^{k}
Average	**2.3±0.2** ^{A}	**1.4±0.2** ^{A,B}	**0.005±0.004** ^{A}	**3.7±0.3** ^{A,B}	**22±9** ^{A}	**70±67** ^{A,B}	**13±7** ^{B}	**297±143** ^{A,B}	**401±185** ^{A}
BPGV11098	2.09±0.02 ^{e,f}	1.0±0.1 ^{i,j}	nd	3.1±0.1 ^{f}	12.3±0.3 ^{o}	39.1±0.2 ^{i}	14.64±0.02 ^{j}	186±7 ^{l}	252±7 ^{j}
BPGV11350	2.120±0.001 ^{d,e}	0.62±0.03 ^{k}	0.032±0.001 ^{a}	2.77±0.03 ^{g}	46±1 ^{b}	57±2 ^{f}	10.9±0.2 ^{l}	356.8±0.2 ^{f}	470±1 ^{e,f}
BPGV11696	2.170±0.005 ^{d,e}	1.52±0.01 ^{c,d}	nd	3.70±0.01 ^{c}	19.3±0.2 ^{k,l}	58±1 ^{f}	23.2±0.1 ^{b}	481±2 ^{b}	582±3 ^{b}
BPGV12465	1.94±0.04 ^{g}	1.25±0.03 ^{f,g}	nd	3.20±0.07 ^{e,f}	26.1±0.3 ^{f}	72±1 ^{e}	6.7±0.1 ^{o}	592±7 ^{a}	697±7 ^{a}
BPGV13034	2.009±0.004 ^{f,g}	1.25±0.01 ^{f,g,h}	0.007±0.003 ^{b,c,d}	3.260±0.001 ^{e,f}	41.0±0.4 ^{c}	29.2±0.7 ^{k}	11.54±0.04 ^{k}	380±3 ^{e}	462±2 ^{f}
Average	**2.06±0.09** ^{B}	**1.1±0.3** ^{B}	**0.01±0.01** ^{A}	**3.2±0.3** ^{C}	**29±13** ^{A}	**51±16** ^{A,B}	**13±6** ^{B}	**399±140** ^{A}	**492±153** ^{A}
BPGV11465	1.81±0.05 ^{h}	0.7±0.1 ^{k}	nd	2.53±0.04 ^{h}	31±1 ^{e}	218±1 ^{a}	11.60±0.01 ^{k}	162±3 ^{m}	422±6 ^{g}
BPGV11907	2.49±0.05 ^{a,b}	1.61±0.02 ^{b,c}	0.010±0.001 ^{b}	4.10±0.1 ^{b}	16.86±0.06 ^{m}	35.0±0.6 ^{j}	17.1±0.1 ^{g}	227±5 ^{k}	296±3 ^{i}
BPGV12260	2.410±0.001 ^{b,c}	1.67±0.02 ^{b}	0.002±0.001 ^{c,d,e}	4.08±0.02 ^{b}	20.4±0.5 ^{j,k}	127.2±0.9 ^{c}	15.5±0.1 ^{i}	269±6 ^{i,j}	432±4 ^{g}
BPGV12437	1.96±0.03 ^{g}	1.36±0.05 ^{e,f}	0.002±0.001 ^{d,e}	3.33±0.08 ^{d,e}	21.8±0.2 ^{i,j}	49.2±0.3 ^{h}	16.63±0.02 ^{h}	288±3 ^{h}	376±3 ^{h}
BPGV12506	1.73±0.02 ^{h}	0.98±0.02 ^{j}	nd	2.71±0.04 ^{g}	89±1 ^{a}	40.9±0.1 ^{i}	17.9±0.1 ^{f}	395±7 ^{e}	543±7 ^{c}
Average	**2.1±0.3** ^{B}	**1.3±0.3** ^{A,B}	**0.002±0.003** ^{A}	**3.3±0.7** ^{B,C}	**36±28** ^{A}	**94±73** ^{A}	**16±2** ^{B}	**268±79** ^{B}	**414±83** ^{A}
BPGV11400	2.37±0.01 ^{c}	1.61±0.01 ^{b,c}	0.010±0.001 ^{b}	3.99±0.02 ^{b}	15.0±0.6 ^{n}	41.490±0.003 ^{i}	27.4±0.2 ^{a}	284±1 ^{h,i}	368±2 ^{h}
BPGV11681	2.47±0.04 ^{a,b}	1.83±0.02 ^{a}	nd	4.30±0.06 ^{a}	18.1±0.1 ^{l,m}	23.5±0.5 ^{l}	21.2±0.2 ^{d}	433±11 ^{d}	496±12 ^{d}
BPGV11803	2.17±0.04 ^{d,e}	1.13±0.04 ^{h,i}	0.0100±0.0001 ^{b,c,d}	3.31±0.01 ^{d,e}	22.4±0.3 ^{h,i}	28.7±0.2 ^{k}	17.380±0.005 ^{g}	227±8 ^{k}	296±8 ^{i}
Average	**2.3±0.1** ^{A}	**1.5±0.3** ^{A}	**0.006±0.004** ^{A}	**3.9±0.4** ^{A}	**19±3** ^{A}	**31±8** ^{B}	**21±4** ^{A}	**315±92** ^{A,B}	**387±88** ^{A}

In each column, different lowercase letters indicate that at least one tomato accession differs statistically ($p < 0.05$) from the others, while different capital letters indicate that at least one of the four groups of tomato accessions established by the PCoA differs statistically ($p < 0.05$) from the others.

Oxalic, malic, ascorbic, and citric acids were identified in the studied tomato accessions (Table 5). Citric acid was the most abundant organic acid in all genotypes, with values ranging from 58 mg 100 g^{-1} in salad tomato (BPGV16388) to 592 mg 100 g^{-1} in "sem varas" tomato (BPGV12465). Similar values (320, 336 and 354 mg 100 g^{-1}, mean values) were described for a collection of 28 genotypes of "long storage" tomato from the Southern Italy (Siracusa et al., 2018), 69 accessions of local tomato varieties from the region of Valencia, Spain (Figàs et al., 2015), and 5 tomato cultivars harvested in Tenerife, Canary Islands (Suárez et al., 2008), respectively.

Malic and oxalic acids were classified as being the second and third most abundant organic acids after citric acid, with concentrations

ranging from traces (tr) to 218 mg 100 g^{-1} and 8.55–89 mg 100 g^{-1}, respectively, in accordance to the results of other studies (Fernández-Ruiz et al., 2004; Suárez et al., 2008). Among the analyzed genotypes, BPGV16388 and BPGV12465 contained the lowest (58 mg 100 g^{-1}) and highest (592 mg 100 g^{-1}) levels of citric acid, respectively, and also of total organic acids (73–697 mg 100 g^{-1}) (Table 5). In addition to the organic acids identified in this study, fumaric and pyruvic acids have been reported in tomato cultivars from Tenerife (Suárez et al., 2008).

Ascorbic acid levels greater than ~18 mg 100 g^{-1} were quantified in the accessions BPGV12506, BPGV12954, BPGV11681, BPGV11363, BPGV11696, and BPGV11400 (Table 5), whose 100-g portions contain more than 20% of the RDA of vitamin C (90 and 75 mg day^{-1} for male and female adults aged 19 or more years old, respectively (Otten et al., 2006)). In addition, the contribution of the last three referred accessions to the RDA of this vitamin for adult women is higher than 30%. In general, the detected levels of ascorbic acid are in agreement with those previously described for tomato cultivars from Tenerife (6.8–27.4 mg 100 g^{-1}; Suárez et al. (2008)) and traditional varieties from North-eastern Portugal (10.8–18.5 mg 100 g^{-1}; Pinela et al. (2012)), but lower than those reported by Figàs et al. (2015) for some varieties from the region of Valencia (12.79–37.34 mg 100 g^{-1}). Much higher values of ascorbic acid were attributed to varieties from Almeria (39–263 mg 100 g^{-1}) (Guil-Guerrero and Rebolloso-Fuentes, 2009); however, the contents of this vitamin may have been overestimated, since a colorimetric method was used for quantification.

When comparing the four clusters, significant differences ($p < 0.05$) can be observed for citric, malic, and ascorbic acids (Table 5). In general, samples from the top-right quartile of the PcoA biplot (*e.g.*, BPGV12465) contained more citric acid and fewer total sugars. On the other hand, the cluster with more free sugars tended to contain more ascorbic acid and less malic acid. These differences in sugars and acids probably reflect variations in tomato organoleptic characteristics.

3.3.3. Composition in fatty acids, tocopherols, carotenoids, and chlorophylls

The most abundant fatty acids in the studies tomato genotypes are shown in Table 6, as well as the categories of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). Nineteen fatty acids were identified in most of the samples (data not shown) and the most abundant were palmitic acid (C16:0, from

Table 6
Composition in fatty acids of the ripen fruits of the BPGV tomato accessions clustered according the PCoA.

Accession	Major fatty acids (relative %)									Categories		
	C16:0	C18:0	C18:1n9	C18:2n6	C18:3n3	C20:0	C22:0	C23:0	C24:0	SFA	MUFA	PUFA
BPGV11363	43.2±0.1	7.34 ±0.03	5.15 ±0.01	24.21 ±0.06	7.24 ±0.04	1.96 ±0.06	2.03 ±0.02	1.26 ±0.02	2.54 ±0.04	62.86 ±0.06 ^{c}	5.24±0.01 ^{o}	31.91 ±0.07 ^{n}
BPGV11372	39.50 ±0.07	8.17 ±0.01	10.41 ±0.05	25.18 ±0.02	5.11 ±0.02	3.02 ±0.02	1.82 ±0.04	1.19 ±0.06	2.20 ±0.07	58.88 ±0.12 ^{d}	10.51 ±0.08 ^{j}	30.61 ±0.04 ^{o}
BPGV12906	26.1±0.1	6.69 ±0.01	12.4 ±0.04	38.37 ±0.01	7.94 ±0.03	1.92 ±0.02	3.28 ±0.02	0.518 ±0.006	0.541 ±0.009	39.7 ±0.01 ^{j}	13.73 ±0.02 ^{b}	46.58 ±0.04 ^{h}
BPGV12954	44.18 ±0.01	6.49 ±0.02	4.53 ±0.05	24.85 ±0.01	7.11 ±0.03	2.47 ±0.02	2.24 ±0.06	1.14 ±0.01	2.56 ±0.02	63.0±0.1 ^{c}	4.71±0.04 ^{p}	32.30 ±0.05 ^{m}
BPGV16388	22.71 ±0.01	5.446 ±0.002	10.96 ±0.03	48.77 ±0.04	8.11 ±0.05	1.09 ±0.01	0.71 ±0.01	0.123 ±0.003	0.443 ±0.005	31.84 ±0.05 ^{n}	11.12 ±0.03 ^{g}	57.04 ±0.01 ^{b}
Average	**35±9** ^{A}	**6.8±0.9** ^{A}	**9±3** ^{A}	**32±10** ^{A}	**7±1** ^{A}	**2.1±0.7** ^{A}	**1.4±0.9** ^{A}	**0.8±0.5** ^{A}	**1.7±0.9** ^{A}	**51±13** ^{A}	**9±4** ^{A}	**40±11** ^{A}
BPGV11098	27.8±0.1	5.83 ±0.03	7.125 ±0.001	40.63 ±0.02	11.84 ±0.07	1.587 ±0.002	1.35 ±0.02	0.62 ±0.03	1.30 ±0.05	40.30 ±0.05 ^{i}	7.23±0.01 ^{m}	52.47 ±0.05 ^{f}
BPGV11350	35.19 ±0.05	7.22 ±0.03	11.30 ±0.01	31.66 ±0.07	7.101 ±0.005	1.56 ±0.04	1.31 ±0.03	0.80 ±0.02	1.45 ±0.02	49.53 ±0.07 ^{f}	11.38 ±0.01 ^{f}	39.09 ±0.08 ^{i}
BPGV11696	34.05 ±0.01	7.16 ±0.01	12.74 ±0.02	32.99 ±0.08	5.15 ±0.01	2.14 ±0.03	0.86±.01	0.66 ±0.01	1.16 ±0.01	48.78 ±0.07 ^{g}	13.08 ±0.02 ^{c}	38.14 ±0.09 ^{j}
BPGV12465	32.0±0.1	8.82 ±0.01	16.08 ±0.01	33.50 ±0.07	3.07 ±0.01	2.66 ±0.05	0.762 ±0.001	0.407 ±0.006	0.982 ±0.003	47.04 ±0.06 ^{h}	16.19 ±0.01 ^{a}	36.77 ±0.05 ^{k}
BPGV13034	23.925 ±0.001	5.91 ±0.01	10.17 ±0.01	43.87 ±0.01	8.75 ±0.02	1.422 ±0.001	2.38 ±0.01	0.57 ±0.02	0.569 ±0.006	36.01 ±0.04 ^{k}	11.05 ±0.02 ^{h}	56.9±0.1 ^{e}
Average	**31±4** ^{A}	**7±1** ^{A}	**11±3** ^{A}	**37±5** ^{A}	**7±3** ^{A}	**1.9±0.5** ^{A}	**1.3±0.6** ^{A}	**0.6±0.1** ^{A}	**1.1±0.3** ^{A}	**44±5** ^{A}	**12±3** ^{A}	**44±7** ^{A}
BPGV11465	21.5±0.1	4.65 ±0.02	11.98 ±0.02	49.5±0.1	8.75 ±0.02	1.02 ±0.02	0.57 ±0.02	0.28 ±0.01	0.58 ±0.01	29.5±0.1 ^{o}	12.15 ±0.02 ^{d}	58.36 ±0.14 ^{a}
BPGV11907	39.02 ±0.05	7.95 ±0.03	10.85 ±0.02	28.25 ±0.09	5.55 ±0.07	2.60 ±0.01	0.94 ±0.01	0.83 ±0.04	1.47 ±0.03	55.0±0.1 ^{e}	10.95 ±0.02 ^{i}	34.1±0.2 ^{l}
BPGV12260	23.8±0.2	5.359 ±0.002	11.3 ±0.04	43.1±0.1	11.50 ±0.01	0.80 ±0.01	1.635 ±0.003	0.54 ±0.05	0.33 ±0.02	33.5±0.2 ^{m}	11.60 ±0.03 ^{e}	54.9±0.1 ^{d}
BPGV12437	35.67 ±0.04	4.93 ±0.01	3.40 ±0.01	33.9±0.1	13.01 ±0.02	1.24 ±0.08	2.82 ±0.08	0.974 ±0.002	0.81 ±0.03	48.7±0.1 ^{g}	3.983 ±0.001 ^{q}	47.33 ±0.08 ^{g}
BPGV12506	44.71 ±0.05	8.36 ±0.06	6.41 ±0.01	22.52 ±0.03	4.36 ±0.05	3.98 ±0.02	2.00 ±0.03	1.56 ±0.02	2.45 ±0.08	66.16 ±0.1 ^{b}	6.51±0.01 ^{n}	27.34 ±0.09 ^{p}
Average	**33±9** ^{A}	**6±2** ^{A}	**9±3** ^{A}	**35±10** ^{A}	**9±3** ^{A}	**2±1** ^{A}	**1.6±0.8** ^{A}	**0.8±0.4** ^{A}	**1.1±0.8** ^{A}	**47±14** ^{A}	**9±3** ^{A}	**44±12** ^{A}
BPGV11400	47.29 ±0.01	9.20 ±0.04	7.56 ±0.01	17.52 ±0.04	3.95 ±0.06	4.14 ±0.03	2.015 ±0.003	1.66 ±0.03	2.80 ±0.02	70.33 ±0.01 ^{a}	7.63±0.01 ^{l}	22.04 ±0.01 ^{q}
BPGV11681	33.96 ±0.01	7.14 ±0.01	12.70 ±0.02	32.90 ±0.08	5.14 ±0.01	2.14 ±0.03	0.86 ±0.01	0.66 ±0.01	1.16 ±0.01	48.65 ±0.07 ^{g}	13.04 ±0.02 ^{c}	38.31 ±0.09 ^{j}
BPGV11803	24.13 ±0.01	5.30 ±0.03	8.83 ±0.01	44.79 ±0.07	11.60 ±0.02	1.343 ±0.004	0.98 ±0.01	0.390 ±0.002	1.14 ±0.03	34.56 ±0.06 ^{l}	9.06±0.02 ^{k}	56.38 ±0.05 ^{c}
Average	**35±10** ^{A}	**7±2** ^{A}	**10±2** ^{A}	**32±12** ^{A}	**6.7±4** ^{A}	**3±1** ^{A}	**1.3±0.6** ^{A}	**0.9±0.6** ^{A}	**1.7±0.8** ^{A}	**51±16** ^{A}	**10±2** ^{A}	**40±15** ^{A}

Palmitic acid (C16:0); stearic acid (C18:0); oleic acid (C18:1n9); linoleic acid (C18:2n6); α-linolenic acid (C18:3n3); arachidic acid (C20:0); behenic acid (C22:0); tricosanoic acid (C23:0); lignoceric acid (C24:0); SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. In each column, different lowercase letters indicate that at least one tomato accession differs statistically ($p < 0.05$) from the others, while different capital letters indicate that at least one of the four groups of tomato accessions established by the PCoA differs statistically ($p < 0.05$) from the others.

21.5% in BPGV11465 to 47.29% in BPGV11400), linoleic acid (C18:2n6, from 17.52% in BPGV11400 to 49.5% in BPGV11465), oleic acid (C18:1n9, 3.40% in BPGV12437 to 16.08% in BPGV12465), stearic acid (C18:0, from 4.65% in BPGV11465 to 9.20% in BPGV11400), and α-linolenic acid (C18:3n3, from 3.07% in BPGV12465 to 13.01% in BPGV12437). Although there were no differences in the fatty acid profile among the four germplasm clusters established by the PCoA, there were differences ($p < 0.05$) among tomato accessions. BPGV16388 and BPGV11465 were particularly rich in PUFA (> 57%) and had a low percentage of SFA (< 32%), while MUFA were abundant in BPGV12906 and BPGV12465 (≥ 13.73%). Therefore, the salad (BPGV16388) and red (BPGV11465) tomato genotypes were characterized by a healthy fatty acid profile (due to C18:2n6, C18:1n9, and C18:3n3) whose ingestion has been associated with beneficial effects on consumer's health. The fatty acid profile of these two accessions is similar to that described for some Spanish and Portuguese tomato varieties (Guil-Guerrero and Rebolloso-Fuentes, 2009; Pinela et al., 2012), but with lower percentages of 18:1n9.

The four tocopherol isoforms were detected in all the analyzed genotypes (Table 7). The α-tocopherol was the most abundant isoform (reaching 381 μg 100^{-1} g in BPGV12954, corresponding to 90% of total tocopherol content), followed by γ-tocopherol (with 181 μg 100 g^{-1} quantified in BPGV16388, corresponding to ~33% of the total content) (except for BPGV11372 and BPGV12465 in which γ-tocopherol was the most abundant). These profiles are in agreement with those previously described for round and oxheart tomato accessions from Miranda do Douro (although they had ~1.8 times more α-tocopherol than BPGV12954) (Pinela et al., 2012). The USDA database (USDA 2016) also presents α-tocopherol as the major isoform (540 μg 100 g^{-1}) in tomato, followed by γ-tocopherol (120 μg 100 g^{-1}). The salad tomato (BPGV16388) presented the highest levels of total tocopherols (541 μg 100 g^{-1}) and also of β-, γ-, δ-tocopherols, while the lowest concentration (135 μg 100 g^{-1}) was found in the yellow tomato (BPGV12437). Frusciante et al. (2007) studied tomatoes grown in the region of San Marzano, Italy, and quantified vitamin E levels between 170 and 600 μg 100 g^{-1}. These discrepancies may be related to the variety under analysis, physiological state of the plant, and environmental stressors such as water availability, light intensity, temperature, and salinity, etc., factors that may influence the production of reactive species in the plant tissues and lead to consequent production of antioxidant defenses such as tocopherols (Munné-Bosch, 2005; Raiola et al., 2015). Interestingly, the samples with the highest total tocopherol contents (BPGV11465 and BPGV16388) were also rich in PUFA (Table 6), which promote the nutritional value of these genotypes. Furthermore, tocopherols are powerful lipophilic antioxidants capable of protecting unsaturated fatty acids from oxidation (Loyola et al., 2012; Pinela et al., 2016). As observed for fatty acids, the genetic distance that characterized the studied genotypes (Fig. 1B) also did not seem to be associated with variations in the tocopherols content (Table 7).

Lycopene is the pigment responsible for the characteristic red color of tomatoes (Flores et al., 2017; Pinela et al., 2016, 2012). Table 7 shows the lycopene and β-carotene levels quantified in the 18 tomato accessions and the mean values of each cluster defined based on the PCoA biplot (Fig. 1B). The lycopene content varied from ~0.1 mg 100 g^{-1} in yellow tomato (BPGV12437) to ~0.9 in salad (BPGV16388) and oxheart (BPGV12260) tomatoes. Levels ranging from 0.88 to 14.1 mg 100 g^{-1} were already reported for other red tomato varieties (Clinton, 1998; Figàs et al., 2015; Flores et al., 2017). Several studies have shown that the consumption of lycopene-rich foods decreases the risk of chronic diseases, namely cardiovascular diseases and various types of cancer (Friedman, 2013; Pinela et al., 2016). In red tomatoes, β-carotene is generally detected at a lower concentration than lycopene (Figàs et al.,

Table 7
Composition in tocopherols, carotenoids and chlorophylls of the ripen fruits of the BPGV tomato accessions clustered according the PCoA.

Accession	Tocopherols (μg 100 g^{-1})					Pigments (mg 100 g^{-1})			
	α-Tocopherol	β-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total	Lycopene	β-Carotene	Chlorophyll a	Chlorophyll b
BPGV11363	256±5 ^{f}	11.0±0.2 ^{e}	38±2 ^{k}	3.5±0.2 ^{g,h,i}	309±3 ^{f}	0.86±0.01 ^{b}	0.87±0.05 ^{b}	0.050±0.001 ^{i}	0.053±0.001 ^{j}
BPGV11372	53.3±0.5 ^{n}	5.62±0.03 ^{i}	90±3 ^{f}	6.49±0.06 ^{d}	155±3 ^{l}	0.246±0.001 ^{h}	0.27±0.01 ^{j,k}	0.094±0.002 ^{g}	0.135±0.003 ^{f}
BPGV12906	233.9±0.5 ^{g}	11.162±0.001 ^{e}	170.6±0.3 ^{b}	6.1±0.3 ^{d,e}	422±1 ^{d}	0.87±0.01 ^{b}	0.60±0.03 ^{e}	0.154±0.001 ^{d,e}	0.202±0.003 ^{d,e}
BPGV12954	381±3 ^{a}	12.42±0.04 ^{c,d}	28±1 ^{l}	1.52±0.07 ^{k}	423±4 ^{c,d}	0.77±0.01 ^{e}	0.73±0.04 ^{c}	0.053±0.003 ^{i}	0.070±0.005 ^{i,j}
BPGV16388	333±5 ^{c}	15.9±0.7 ^{a}	181±6 ^{a}	10.320±0.003 ^{a}	541±1 ^{a}	0.90±0.03 ^{a}	0.61±0.03 ^{e}	0.21±0.04 ^{b}	0.28±0.01 ^{c}
Average	**251±116** ^{A}	**11±3** ^{A}	**102±67** ^{A}	**6±3** ^{A}	**370±135** ^{A}	**0.7±0.3** ^{A}	**0.6±0.2** ^{A}	**0.11±0.06** ^{A}	**0.15±0.09** ^{A}
BPGV11098	321±3 ^{d}	13.4±0.2 ^{b}	46.3±0.5 ^{j}	3.2±0.1 ^{i}	384±4 ^{e}	0.80±0.01 ^{d}	0.67±0.03 ^{d}	0.10±0.01 ^{g}	0.13±0.01 ^{f}
BPGV11350	131±1 ^{k}	7.6±0.3 ^{g}	61±1 ^{i}	5.0±0.1 ^{f}	204.4±0.3 ^{k}	0.1914 ±0.0005 ^{j}	0.313±0.003 ^{j}	0.073±0.001 ^{h}	0.071±0.002 ^{i}
BPGV11696	194±3 ^{h,i}	10.2±0.2 ^{f}	69.1±0.9 ^{g,h}	3.9±0.2 ^{g}	277±2 ^{g}	0.168±0.001 ^{k}	0.192±0.001 ^{l}	0.061±0.002 ^{h,i}	0.091±0.003 ^{g,h}
BPGV12465	71±3 ^{m}	6.6±0.3 ^{h}	154±2 ^{c}	8.1±0.1 ^{c}	240±5 ^{j}	0.36±0.01 ^{g}	0.40±0.01 ^{i}	0.06±0.01 ^{h,i}	0.09±0.02 ^{g}
BPGV13034	190±3 ^{i}	10.6±0.2 ^{e,f}	63.8±2 ^{l}	3.7±0.2 ^{g,h}	268±3 ^{h}	0.776±0.003 ^{e}	0.51±0.02 ^{g}	0.186±0.003 ^{c}	0.30±0.01 ^{b}
Average	**181±86** ^{A}	**10±3** ^{A}	**79±40** ^{A}	**5±2** ^{A}	**275±62** ^{A}	**0.5±0.3** ^{A,B}	**0.4±0.2** ^{A,B}	**0.09±0.05** ^{A}	**0.14±0.09** ^{A}
BPGV11465	336±5 ^{c}	11.1±0.5 ^{e}	167±2 ^{b}	5.8±0.2 ^{e}	521±8 ^{b}	0.84±0.02 ^{c}	0.55±0.03 ^{f}	0.13±0.01 ^{f}	0.19±0.01 ^{e}
BPGV11907	291±3 ^{e}	13.1±0.5 ^{b,c}	73±1 ^{g}	3.4±0.3 ^{h,i}	380±4 ^{e}	0.77±0.01 ^{e}	0.64±0.02 ^{d,e}	0.075±0.005 ^{h,i}	0.08±0.02 ^{g,h,i}
BPGV12260	263±3 ^{f}	13.431±0.001 ^{b}	104±3 ^{d}	5.682±0.001 ^{e}	387±5 ^{e}	0.91±0.01 ^{a}	0.62±0.03 ^{e}	0.168±0.002 ^{c,d}	0.28±0.02 ^{c}
BPGV12437	75±2 ^{m}	7.4±0.2 ^{g}	49±1 ^{j}	2.700±0.002 ^{j}	135±4 ^{m}	0.106±0.004 ^{l}	1.03±0.03 ^{a}	0.224±0.006 ^{a}	0.36±0.01 ^{a}
BPGV12506	114±4 ^{l}	6.0±0.2 ^{h,i}	73±1 ^{g}	5.0±0.2 ^{f}	198±3 ^{k}	0.212±0.005 ^{i}	0.28±0.01 ^{j,k}	0.05±0.01 ^{i}	0.07±0.01 ^{i,j}
Average	**216±106** ^{A}	**10±3** ^{A}	**93±42** ^{A}	**5±1** ^{A}	**324±145** ^{A}	**0.6±0.4** ^{A,B}	**0.6±0.3** ^{A}	**0.13±0.07** ^{A}	**0.2±0.1** ^{A}
BPGV11400	199.6±17 ^{h}	13.24±0.03 ^{b}	65±3 ^{h,i}	5.20±0.05 ^{f}	283±4 ^{g}	0.375±0.001 ^{g}	0.196±0.002 ^{l}	0.138±0.002 ^{e,f}	0.211±0.003 ^{d}
BPGV11681	141±5 ^{j}	9.9±0.5 ^{f}	99±3 ^{e}	9.3±0.4 ^{b}	258±3 ^{i}	0.170±0.001 ^{k}	0.243±0.004 ^{k}	0.065±0.004 ^{h,i}	0.08±0.01 ^{h,i}
BPGV11803	345.6±0.5 ^{b}	12.2±.0.2 ^{d}	70±2 ^{g}	2.5±0.1 ^{j}	431±3 ^{c}	0.62±0.01 ^{f}	0.470±0.005 ^{h}	0.13±0.01 ^{f}	0.20±0.01 ^{d,e}
Average	**229±102** ^{A}	**12±1** ^{A}	**78±16** ^{A}	**5±2** ^{A}	**324±81** ^{A}	**0.4±0.2** ^{B}	**0.3±0.1** ^{B}	**0.11±0.04** ^{A}	**0.16±0.07** ^{A}

In each column, different lowercase letters indicate that at least one tomato accession differs statistically ($p < 0.05$) from the others, while different capital letters indicate that at least one of the four groups of tomato accessions established by the PCoA differs statistically ($p < 0.05$) from the others.

2015); however, this was not verified for all genotypes (Table 7). The oxheart tomato accession (BPGV11363) from Santarém, for example, had a high β-carotene content (0.87 mg 100 g^{-1}) and a relatively equal amount of lycopene. This difference was more pronounced in BPGV11400, BPGV13034, BPGV11465, and BPGV16388, where lycopene represented ≥ 60% of the sum of both carotenoids. The β-carotene values herein described are comparable to those previously reported by Figàs et al. (2015). Table 7 also shows that the carotenoid contents differentiate the tomato accessions grouped into the four clusters. In general, while samples from the top-left quartile of the PCoA biplot (Fig. 1B) tended to contain slightly more carotenoids, those from the bottom-left quartile had lower concentrations.

Regarding chlorophylls *a* and *b* (Table 7), the yellow tomato BPGV12437 presented the highest contents (0.224 and 0.36 mg 100 g^{-1}, respectively). On the other hand, three of the oxheart tomato accessions (BPGV11363, BPGV12506, and BPGV12954) were characterized by lower chlorophyll *a* and *b* values (≤ 0.05 and 0.07 mg 100 g^{-1}, respectively). In a previous study, Kozukue and Friedman (2003) reported 0.1 mg 100 g^{-1} of chlorophyll $a + b$ in ripe tomatoes harvested 50 days after flowering, and showed that these pigments are enzymatically degraded during fruit ripening while lycopene and β-carotene are synthesized.

3.4. Linear discriminant analysis

A linear discriminant analysis (LDA) was performed to ascertain relevant trait's contribution to the overall variation observed among the characterized table tomato accessions. At first, it was evaluated whether the chemical composition of the accessions could be related to their genetic distance. The samples were grouped according to four quartiles of the PCoA biplot in Fig. 1B and all analyzed chemical parameters were considered simultaneously in the analysis. Three discriminant functions were defined by the model, the first justifying 95.3% of the variance and clearly separating the accessions in the top-left quartile of Fig. 1B especially those from in the top-right quartile, while the second function justified 4.1% and separated the accessions in the bottom quartiles from those especially in the top-right quartile (Fig. 2A). However, there was no clear individualization of the accessions in the bottom quartiles. Thus, despite the genetic distance that characterizes them, these two groups were chemically similar. When evaluating chemical variations between genotypes as a function of their heterozygosity (Fig. 1A), it appeared that the greater the heterozygosity, the greater the chemical variance (Fig. 2B). The BPGV11363 accession with the highest heterozygosity distanced itself from the others with medium-high heterozygosity, mainly due to the total sugars and malic acid contents, and from those with low heterozygosity due to the sucrose, ascorbic acid and δ-tocopherol contents.

As presented in Table 1, the ripe tomato fruits were classified according to their size into small, large, intermediate, and very large. Therefore, a LDA was applied to investigate the relationship between this morphological descriptor and the studied chemical parameters. The two functions represented in Fig. 2C accounted for 91.9% of the total variance, which clearly separated the tomato accessions according to their size. Function 1 justified 61.3% of the variance and separate intermediate-sized fruits from the other, differences explained mainly by the γ- and δ-tocopherol and MUFA contents. Function 2 justified 30.5% of the variance and clearly separated small tomatoes (BPGV11907) from the other, especially the larger ones. This was justified mainly by the carbohydrates content, which tended to be higher in BPGV11907. Therefore, it seemed that the size of the studied tomato genotypes may

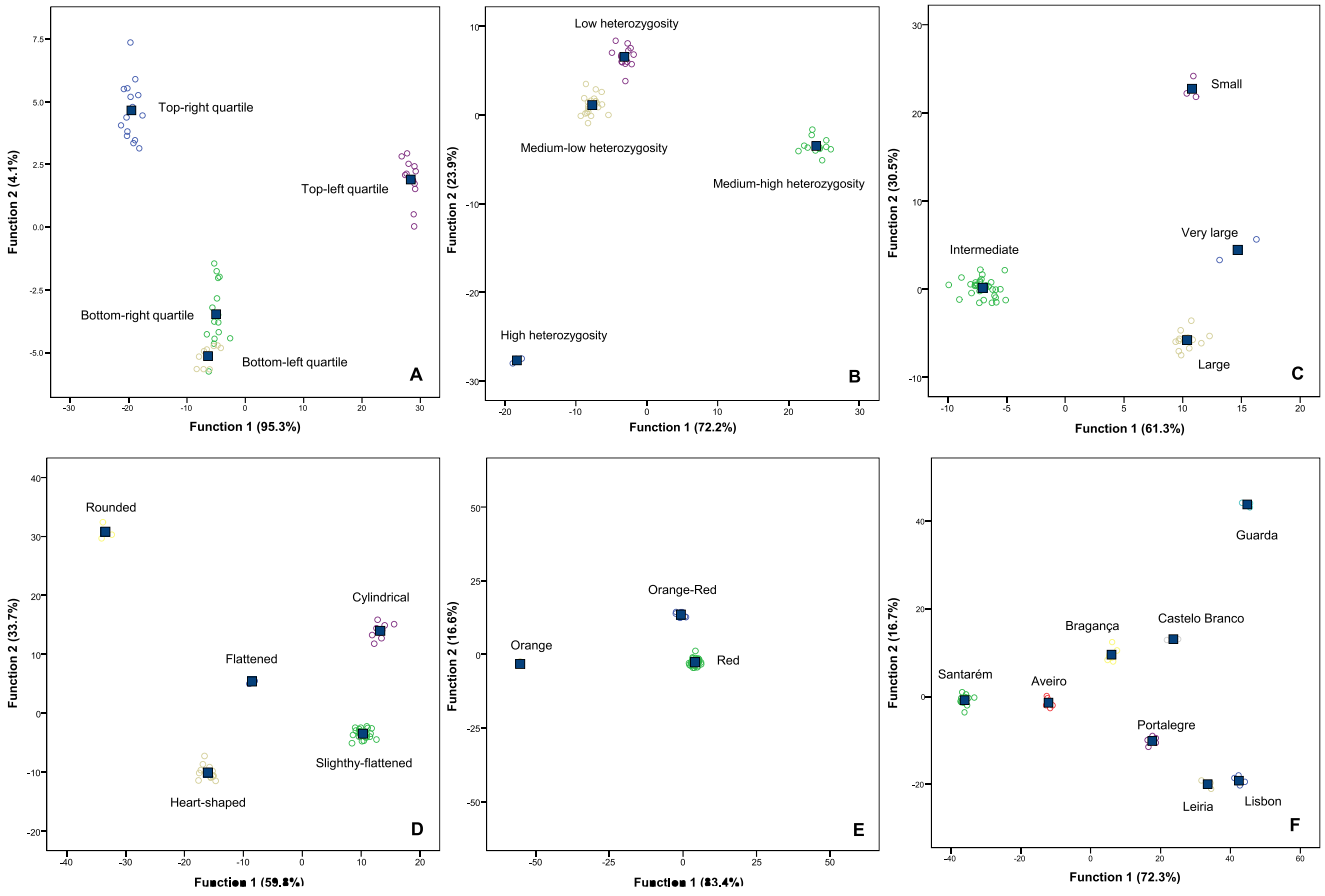

Fig. 2. Spatial distribution set by the canonical discriminant function coefficients when considering as markers the genetic distance between populations (A), the observed heterozygosity (B), the fruit size (C), shape (D), and color (E), and the geographic origin of the accessions (F).

be associated with some distinct compositional features. In turn, Fig. 2D illustrates the spatial distribution of the markers according to the predominant fruit shape (Table 1), where it is possible to verify that rounded shape fruits trended to differ from the other (mainly in the levels of fructose, ascorbic acid, and α- and β-tocopherol). According to Fig. 2E, Function 1 (83.4%) allowed the separation of orange tomatoes from the orange-red and red ones based on protein, C18:3n3, and γ-tocopherol contents, while Function 2 (16.6%) was mainly related to differences in the levels of β-carotene and lycopene, C18:1n9, total tocopherols, citric and ascorbic acids, and fructose.

An LDA was also applied to find out if the geographical origin of the studied tomato accessions can be associated with variations in their chemical composition. The biplot in Fig. 2F illustrates two of the seven canonical discriminant function, which accounted for 89% of the total variance. Function 1 (72.3%) was the most effective in separating the markers corresponding to accessions from Guarda and Lisbon from those from Santarém, while function 2 (16.7%) was effective in separating the marker corresponding to accessions from Guarda (BPGV13034). It was interesting to note that the distribution of the eight markers follows a somewhat interesting trend, since the regions of Lisbon and Leiria are close and on the coast. On the other hand, the Guarda, Castelo Branco, and Bragança are inland regions.

4. Conclusion

Morphological, chemical, and molecular studies are important for the identification and selection of improve tomato genotypes. In this work, 20 important tomato genotypes commonly grown in Portugal were characterized. Maximum morphological and molecular variation was observed in all tested genotypes. A genetic fingerprint consisting of seven pairs of SSR primers has been successfully useful to accurately distinguish the 20 tomato accessions. The correlation between molecular and chemical data showed that the greater the heterozygosity, the greater the compositional variance. Still, the genetic distance that characterized the germplasm did not always reflect chemical differences. Overall, these results can be used to assist the breeding of tomato varieties and contribute to the mainstreaming tomato biodiversity conservation and sustainable use. The use of these varieties in sustainable agricultural systems, as quality, tasty and healthy foods, is also supported and could contribute to the promotion of nutrition and food safety programs better adjusted to the consumers' preferences and dietary needs.

CRediT authorship contribution statement

José Pinela: Investigation, Writing – original draft. **César Montoya:** Investigation. **Valter Martins:** Investigation. **Maria Eugénia Nunes:** Conceptualization, Investigation, Resources, Writing – review & editing. **Filomena Rocha:** Conceptualization, Investigation. **Violeta Rolim Lopes:** Conceptualization, Investigation. **Ana Maria Barata:** Conceptualization, Investigation, Resources, Writing – review & editing. **Ana Maria Carvalho:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Isabel C.F.R. Ferreira:** Conceptualization, Resources. **Lillian Barros:** Investigation, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare no competing financial interest.

Data availability

No data was used for the research described in the article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2023.111938.

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