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Characterization of local tomato varieties (*Solanum lycopersicum* L.) to promote their valorization and identify new production paths

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Abstract

The use of genetic resources plays a key role in the preservation of agrobiodiversity and it will be of significant importance to increase the performance of some of the most productive crops in the world and to boost sustainable agriculture and environmental protection. The conservation and characterization of landraces can contribute to food security and improve food nutrition as they represent an important crop heritage possessing quality and sensory characteristics that consumers and industries esteem. Also, the use of landraces in local markets can contribute to the sustainability of rural communities and satisfy both farmers and consumer demands.

Accordingly, in the present study a collection of tomato (*Solanum lycopersicum* L.) Sardinian landraces were characterized and evaluated in comparison to vintage and modern varieties for evaluating: a) the response of the local varieties to the greenhouse conditions during the autumn/winter season and adopting modern horticultural techniques; b) the diversity of the collection based on morpho-phenological, metabolic and genetic data; c) their response to storage and the changes in their quality characteristics; d) the willingness to pay of consumers for local tomato varieties rather than commercial varieties.

Results revealed that the Sardinian tomato landraces collection is characterized by a high diversity for numerous morpho-phenological and quality traits, allowing to outline the main characteristics that are in a close relationship with their nutritional and commercial value. Moreover, it emerged the consumers willingnes to pay high premium prices for local tomato varieties, demonstrating the increasing attention for quality and sustainable food. All these results could be useful to valorize these local tomato varieties in future breeding programs, plan strategies and programmes to support their cultivation and develop regional and national markets adapt to acknowledge their characteristic.

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CHAPTER 1

1 Phenotypic, genetic and metabolic characterization

1.1 Introduction

Plant genetic resources (PGR) for food and agriculture are the most valuable and basic raw materials to meet the current and future needs of crop improvement programmes (FAO, 2010). The PGR of any crop include modern and vintage cultivars, genetic stocks, breeding lines, landraces, ecotypes and crop wild relatives (FAO, 2010). The loss of these materials, and a consequent decrease of crop genetic diversity, increases the vulnerability of future food productions and food supply with possible related food security issues (Halewood et al., 2017; Lazaro et al., 2018). Therefore, genetic resources have a key role in the resilience and sustainability of agriculture and must be preserved to maintain the overall genetic variability present in a species (McCouch et al., 2013; Casals et al., 2017; Hufford et al., 2019). For decades, international organizations and the scientific community, were increasingly involved in the preservation of genetic resources through collection, characterization (morpho-phenotypic, biochemical and genetic analyzes) and conservation by *ex-situ* methods (conservation outside the natural habitat, such as in gene-banks) and *in-situ* methods (conservation in natural habitat, such as on farm cultivation) of different species of agricultural importance (Gepts, 2006; Tuberosa et al., 2011). Recently, farmers and stakeholders were also encouraged in the conservation and use of plant genetic resources (Lazaro et al., 2018). Plant breeders and gene-banks are widely engaged in the

conservations of PGR, but among them ecotypes and landraces still need to be actively conserved and preserved (Gepts, 2006; Polegri and Negri, 2010).

Vavilov (1887-1943), a Russian geneticist and biologist, was one of the first to draw attention not only to wild relatives but also to landraces as sources of genetic variability, determinant for the future of agriculture. Landraces, also called regional or local varieties, represent the earliest forms of cultivated varieties which have been generally selected by farmers for subsistence agriculture in variable environments where production must be guaranteed. Hence, they are constituted and grown in specific geographical areas adapting to environments and cultural techniques imposed by farmers (Harlan, 1975; Brush, 2000). These populations are highly heterogeneous and represent a precious source of genes that underlie quality and productive traits to be exploited in marginal environments (Harlan, 1975; Brush, 2000; Villa et al., 2005). Both wild species and landraces have a wider genetic variability than modern cultivars and the genes present in these varieties represent a reservoir useful to increase the performance of some of the most productive crops in the world and to boost sustainable agriculture and environmental protection (Harlan, 1975; Brush, 2000; McCouch, 2004; Petropoulos et al., 2019; Hufford et al., 2019).

Fundamental for revealing the differences between local varieties and cultivars and to assess their inherent diversity, is their characterization, which includes morpho-phenological, chemical, organoleptic and molecular strategies (Terzopoulos and Bebeli, 2010; McCouch et al., 2013; Hurtado et al., 2014; Figàs et al., 2015a; Figàs et al., 2018).

The phenotypic characterization is usually performed by conventional morpho-phenological descriptors at different levels such as seedling, plant development, inflorescence, flower and fruit and agronomic traits which are essential to evaluate their performances, identify specific characteristics and

distinguish different landraces or landraces from modern cultivars (Scott, 2010; Hurtado et al., 2014). A detailed phenotypic analysis of germplasm resources is also necessary to register and protect landraces as recognized conservation varieties (Spataro and Negri, 2013). In the last few years, conventional phenotyping has been supported by phenomics (Houle et al., 2010). When conventional descriptors are ineffective in registering the differences among local varieties more precise tools, such as high throughput phenomics softwares (e.g. Tomato Analyzer), might aid in the study of complex traits (Figàs et al., 2015a). Phenomics makes use of non-invasive and automated technologies to capture plant features on the basis of image analysis. This increases the chances of identifying the genetic basis of complex traits and gaining insights into relevant biological phenomena on the basis of different important variables (Houle et al., 2010). This will render plant breeding faster, more affordable and efficient (McCouch et al., 2013).

The characterization of an individual can also be conducted through the detection of chemical and organoleptic characteristics (Hurtado et al., 2014). Indeed, the investigation of relevant nutritional and bioactive compounds could allow to identify outstanding values in particular accessions, indicating those materials that may be used in crop improvement programmes (Hurtado et al., 2014; Kavitha et al., 2014). Among these compounds, metabolites are of considerable interest for the characterization of fruit species (Figàs et al., 2015b; Baldina et al., 2016; Tamasi et al., 2019). In fact, chemical compounds such as pigments, fatty acids, amino acids, flavonoids and volatiles determine the quality of a fruit (Quinet et al., 2019). For instance, most of them regulate the overall flavor of the fruits, characteristic often poor in the modern commercial varieties and the major cause of consumers complaint (Causse et al., 2010; Tieman et al., 2017). Metabolites influence very important marketing factors that affect the buying decision of the industries and consumers (Ilahy et al., 2011). In particular, natural pigments as carotenoids,

anthocyanins and chlorophyll are the major responsible of the green, yellow, orange, pink and red colors of fruits and vegetables (Ruiz-Sola and Rodríguez-Concepcíon, 2012). In tomato, for example the chlorophyll imparts a green color during the early developmental stages of the fruit, then, during the ripening process, it is degraded in the transition from chloroplasts to chromoplasts with a consequent increase of lycopene, which imparts the red color to the fruits, and β-carotene (Fraser et al., 1994; Rosati et al., 2000; Adalid et al., 2010). The quali-quantitative characterization of many chemicals is also important to define the dynamics of the biosynthetic processes during the ripening of fleshy fruits (Giovannoni et al., 2017; Kozukue and Friedman, 2005; Llorente et al., 2016). Indeed, the metabolic changes generally associated with the accumulation of sugars, acids and volatile compounds occur during ripening, thus determining taste, smell, color and softening of the fruit (Klee and Giovannoni, 2011; Giovannoni, 2007). Metabolites are important not only for the role they play in different physiological and biochemical processes, but also for their nutritional value and the related marketability (Canane-Adams et al., 2005; Giovannoni et al., 2017; Bertin and Génard, 2018; Quinet et al., 2019). Indeed, bioactive compounds such as carotenoids and vitamins, have recognized health benefits and a role in the prevention of several diseases and dysfunctions (e.g. cardiovascular diseases, prostate and esophagus cancer) (Martí et al., 2016; Livingstone et al., 2017; Kulczyński et al., 2017; Abbasi et al., 2019). All these compounds involved in different physiological and biochemical processes, are highly variable in traditional germplasm. As a consequence, their study represents an effective method for the identification of genetic resources that can be exploited in breeding programs for the production of new commercial cultivars with target traits (Figàs et al., 2015b; Baldina et al., 2016; Petropoulos et al., 2019).

An efficient characterization and conservation of landraces and a consequent development of future strategies for a sustainable use of crops or

for their improvement, also require a genetic characterization and an estimation of their genetic diversity (Lobate et al., 2011; McCouch et al., 2013; García-Martínez et al., 2013). Numerous studies have been conducted and numerous methods have been defined to evaluate the genetic diversity of the cultivated species, also based on the combined analysis of phenotypic traits and molecular markers (Terzopoulos and Bebeli, 2008; Mazzucato et al., 2010; García-Martínez et al., 2013). Various molecular techniques are used to estimate the genetic diversity of a germplasm collection and distinguish the genotypes within a population. Single Nucleotide Polymorphisms (SNPs) are the most frequently used among many others developed in the past, including restricted fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR) and sequence-related amplified polymorphism (SRAP) (Miller and Tanksley, 1990; Paran et al., 1995; Carelli et al., 2006; Mazzucato et al., 2008; Davey et al., 2011; Cebolla-Cornejo et al., 2013; Al Shaye et al., 2018). The increased availability of modern and sophisticated technologies for genomic investigations has allowed to collect huge numbers of genetic markers. Indeed, the advent of next generation sequencing (NGS) technologies has reduced the time and the cost of sequencing, SNPs discovery and genotyping (Ray and Satya, 2014; D'Agostino and Tripodi, 2017). Among them, genotyping-by-sequencing (GBS) has emerged as an efficient genomic approach for quick genotyping and exploration of plant genetic diversity (Taranto et al., 2016).

In accordance with the premise, in this study, a collection of tomato Sardinian landraces has been characterized and evaluated under greenhouse conditions in comparison to commercial varieties, with the aim to assess the diversity of the collection based on morpho-phenological, metabolic and genetic data. In detail, one objective was to determine the suitability of the local tomato varieties to greenhouse conditions during the autumn/winter

season under modern horticultural techniques. These landraces are usually cultivated by local farmers in open-field using a conventional management. In parallel, an in-depth characterization of these varieties through investigation of some nutraceutical proprieties was obtained, focusing on the detection of primary metabolites and on selected secondary metabolites (carotenoids) at two ripening stages of the fruit (breaker and ripe stage). The genetic diversity level of the collection was also assessed by means of genomic SNP markers. The results here obtained could be useful to the enhancement of these local products, promote their direct use in local markets and valorize their peculiarities in different contexts, such as in future breeding programs.

1.2 Materials and Methods

1.2.1 Plant Materials and experimental design

Plant materials consisted of a collection of tomato (*Solanum lycopersicum* L.) Sardinian landraces, vintage and modern varieties. The Sardinian landraces were mainly collected during 2006 and 2007 (Attene and Rodriguez, 2008) if the farmer declared that he had cultivated and multiplied the seeds for at least thirty years; an assumption on which a cultivated variety can be considered local (Louette, 2000). Seeds of Sardinian landraces are stored at the "Centro Interdipartimentale per la Conservazione e Valorizzazione della Biodiversità Vegetale" (CBV), University of Sassari, Italy (Attene et al., 2015). The vintage variety, named "Varrone", was created in the early 1900s by Nazareno Strampelli (Italian agricultural geneticist) (Salvi et al., 2016). The commercial varieties were chosen from those commonly cultivated and marketed during the autumn/winter season by the farmer partner of the project. The three commercial varieties, used as a control in the trials, are representative of the different typologies of varieties evaluated in this

research and all cultivated for fresh consumption: the "Tombola" variety is characterized by flatten/slightly flatten shaped fruit with large dimensions; the "Camone" variety possess rounded and medium/small-sized fruits; while the "Datterino" variety is characterized by small and elongated fruits.

A total of 17 accessions were grown under greenhouse conditions during the autumn-winter season for two consecutive years, 2017-2018 and 2018-2019. The trials were conducted on a horticultural specialized farm (Società Agricola F.lli Scintu, 36°55'45.36'' N, 8°37'39.88'' E) located in Oristano, Sardinia, following a randomized complete block design with three replicates. The field was characterized by 5 mulched rows (3 as experimental trial and 2 as borders), with 1.70 m of distance between the rows and plants spaced 0.40 m apart in-the-row. The same cultivation techniques and agronomic practices of the farm have been adopted. The sowing, in pots, was carried out by hand at the end of July in the greenhouse of the Department of Agriculture in the experimental farm "M. Deidda" in Ottava, Sassari. Transplantation of the seedlings was done by hand at the end of August in the Scintu's farm. Plants of commercial tomato varieties were transplanted all around the field as borders of the trials. All plants were staked by cords and clips on two stems (peculiar type of cultivation adopted in the greenhouse), constantly pruned during the growth and, when the plants reached the height of about 1.8 m, the apex was trimmed. The trials ended up in January/February when all fruits were harvested.

In the 2017-2018 experimental trial (EX1), 12 Sardinian landraces, one Italian vintage variety and three modern varieties were evaluated. Data previously collected on a wide collection of local tomato varieties (Scintu, 2014), were used to choose the varieties analyzed in the present study, in a way to be representative of the morpho-phenotypic and molecular variability present within the collection. In particular, the varieties characterized by determined growth habit were excluded from the evaluation and the most

interesting varieties were chosen to represent the different typologyes (e.g. plum, beefsteak, oxheart).

Based on the results obtained from the EX1, six landraces were chosen for the 2018-2019 experimental trial (EX2). An additional local variety (Tamatta groga de appiccai), which was not evaluated in EX1 because of seeds germination problems, the Italian vintage variety and the three commercial varieties evaluated in the first trial were included in the second trial. The list of the varieties analyzed during the two trials is shown in Table 1.

Table 1: List of accessions evaluated in both EX1 and EX2.

Name	Code	Collection[a]	Commercial Category	EX1	EX2
Arracadas	P01	L-SAR	Plum	x	x
Lorighittas	P02	L-SAR	Plum	x	
Tamatticasa tundas a siccu	P04	L-SAR	Beefsteak (round)	x	x
Lorigheddas de appiccai	P05	L-SAR	Plum	x	x
Tamatta siccada	P08	L-SAR	Beefsteak (oblate)	x	
Tamatta kaki	P16	L-SAR	Beefsteak (round)	x	x
Tamatta	P29	L-SAR	Beefsteak (oblate)	x	
A melasa a melasa	P30	L-SAR	Oxheart	x	
Tomattis mannu de Bachis	P33	L-SAR	Beefsteak (oblate)	x	
Butirra	P36	L-SAR	Ribbed	x	x
Tamatta groga de appiccai	P44	L-SAR	Round		x
Tamatta cor'e boi	P46	L-SAR	Oxheart	x	x
Cor'e boi afriscilonada	P52	L-SAR	Oxheart	x	
Varrone	P113	VV	Round	x	x
Camone	C1	CV	Beefsteak (round)	x	x
Tombola	C2	CV	Beefsteak (oblate)	x	x
Datterino	C3	CV	Mini plum	x	x

[a] L-SAR = Sardinian landrace; VV = vintage variety; CV = commercial variety

1.2.2 Phenotypic analysis

In both experimental trials, individual plants were characterized using fifteen agronomic descriptors, both phenological and morphological, including qualitative and quantitative traits. The descriptors were mainly based on the guidelines of the Bioversity International (formerly IPGRI; 1996). The registered descriptors were: days to flowering from sowing date (*DTFs*), days to flowering from transplanting date (*DTFt*), flowering-ripening interval (*FRI*), number of flowers per inflorescence (*NFI*), inflorescence type (*ITP*, score), mean fruit weight (*FW*, g), fruit length (*FLE*, cm), fruit width (*FWI*, cm), fruit length/width (*FL/W*, [*FLE*/*FWI*]), fruit color (*FCO*, score), fruit shape (*FSH*, score), shape of pistil scar (*SPS*, score), fruit blossom end shape (*SBE*, score), fruit cross-sectional shape (*FSS*, score) and number of locules (*NOL*). The parameters scored in each trial are listed in Table 2.

Table 2: List of morpho-phenological traits evaluated in EX1 and EX2.

List of Descriptors	Code	Type[a]
Days to flowering from sowing (days)	DTFs	QNT
Days to flowering from transplanting (days)	DTFt	QNT
Flowering-ripening interval (days)	FRI	QNT
Number of flowers per inflorescence	NFI	QNT
Inflorescence type	ITP	QLT
Mean fruit weight (g)	FWG	QNT
Fruit length (cm)	FLE	QNT
Fruit width (cm)	FWI	QNT
Fruit length/width	FL/W	QNT
Fruit color	FCO	QLT
Fruit shape	FSH	QLT
Shape of pistil scare	SPS	QLT
Fruit blossom end shape	SBE	QLT
Fruit cross-sectional shape	FSS	QLT
Number of locules	NOL	QNT

[a] QNT = Quantitative traits, QLT = Qualitative traits

1.2.3 Molecular analysis

Molecular analysis was performed on all the accessions evaluated during both EX1 and EX2, except for the commercial variety C2 (no seeds availability) (Table 1).

The genomic DNA was extracted from fresh and young leaves by taking approximately 3 g of tissue from four plants per accessions for the landraces (they are pure lines) and from one plant per commercial variety. The tissue was frozen by liquid nitrogen and grinded by mortar and pestle. The DNA was extracted following the next protocol: 1.2 g of ground tissue was washed in 40 ml of pre-wash buffer (ddH_2O, 1 M Tris(hydroxymethyl)aminomethane [Tris], 1 M sorbitol, 0.5 M ethylene-diaminetetraacetic acid [EDTA], polyvinylpyrrolidone 40 [PvP] and β-mercaptoethanol), mixed by shaking and gentle vortex and then centrifuged at 2500 g for 5 min at room temperature. The pre-wash buffer, containing polysaccharides and polyphenols, was poured out and 30 ml of CTAB extraction buffer (1 M Tris(hydroxymethyl)aminomethane [Tris], NaCl, 0.5 M ethylene-diaminetetraacetic acid [EDTA], polyvinylpyrrolidone 40 [PvP], β-mercaptoethanol and hexadecyltrimethylammonium bromide [CTAB]) was added to the pre-washed tissue. The homogenate was incubated at 65°C for 15 min. Following the incubation, 14 ml of chloroform/isoamyl alcohol (24:1) was added and the homogenate was centrifuged for 10 min at 1300 g at room temperature. The aqueous layer was removed and transferred in a new tube, 30 µl of 10 mg/ml RNAase was mixed and then incubated for 30 min at room temperature. Following the incubation, 20 ml of isopropanol was added to precipitate the DNA. The DNA was resuspended in 0.6 ml of TE buffer (10 mM Tris-HCl and 1 mM EDTA). Double-stranded DNA concentrations were quantified using the NanoDrop spectrophotometer (Thermo Fisher Scientific, MA, USA).

Libraries with insert sized of 200-500bp fragmented genomic DNA were constructed according to the manufacturer's instructions and sequenced on an Illumina NovaSeq platform. Sequence reads were aligned against the Heinz 1706 reference genome and 1190886 SNPs were called. The raw datafile (1190886 SNPs) was filtered based on the following criteria: loci with more than 70% of missing data were removed, heterozygous SNPs and INDELS were also excluded from the analysis and a final dataset of 2165 loci was obtained.

1.2.4 Metabolic analyzes

Metabolic analyzes have been performed on the accessions cultivated in EX2. Metabolites were extracted from three replicates of each accession (one per block) both at breaker and red stage (Fig. 1), for a total of 66 samples. Harvested fruits were left for 6 hours on a laboratory bench at room temperature, then cut and cleaned from seeds, frozen with liquid nitrogen and stored at -80°C until extraction.

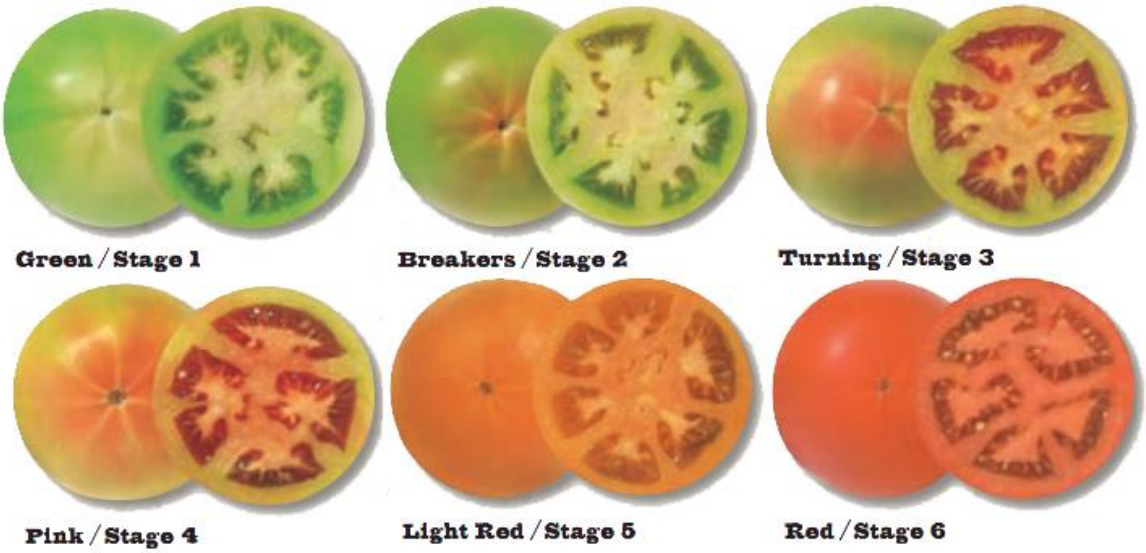


Figure 1: Tomato fruit ripening stage. Source: California Tomato Commission.

The frozen flash samples from each fruit stage were rapidly homogenized and three in one methyl tert-butyl ether (MTBE) extraction protocol (Giavalisco et al., 2009) was used to extract the metabolites. The primary metabolite profiling was performed using a gas chromatography-mass spectrometry (GC-MS) protocol as described in Lisec et al. (2006). Part of the polar fraction from MTBE extraction was dried under vacuum, and the residue was derivatized for 120 min at 37°C (in 40 µl of 20 mg ml-1 methoxyamine hydrochloride in pyridine) followed by a 30 min treatment at 37°C with 70 µl of MSTFA. An autosampler Gerstel Multi-Purpose system (Gerstel GmbH & Co.KG, Mülheim an der Ruhr, Germany) was used to inject the samples to a chromatograph coupled to a time-of-flight mass spectrometer (GC-MS) system (Leco Pegasus HT TOF-MS, LECO Corporation, St. Joseph, MI, USA). Helium was used as carrier gas at a constant flow rate of 2 ml/s and gas chromatography was performed on a 30 m DB-35 column. The injection temperature was 230°C and the transfer line and ion source were set to 250°C. The initial temperature of the oven (85°C) increased at a rate of 15°C/min up to a final temperature of 360°C. After a solvent delay of 180 sec mass spectra were recorded at 20 scans s-1 with m/z 70-600 scanning range. Chromatograms and mass spectra were evaluated by using Chroma TOF 4.5 (Leco) and TagFinder 4.2 software. A total of 87 compounds were detected.

Among the secondary metabolites, a particular attention was given to carotenoids, which were extracted following a specific protocol developed by the Giovannoni laboratory members in the Boyce Thompson Institute for Plant Research (BTI), Cornell University, Ithaca (NY, USA). Briefly: approximately 100 mg of frozen ground tomato fruit tissue were lyophilized with VirTis BenchTop 4K Freeze Dryer (SP Industries Inc., Warminster, PA, USA), then 100 µL of $MgCO_3$ and 500 µL of Ethyl acetate were added, and the samples were shaken. After an incubation of 15 minutes at 4°C, the samples were spun at 13000 rpm for 5 minutes, the clean fraction of supernatant was kept and placed in the

Vacufuge 5301 Centrifugal Vacuum Concentrator (Krackeler Scientific Inc., Albany, NY, USA) for about 15 min or until completely dried. The samples were stored at -80°C until carotenoid detection. All procedures were conducted in a dark room and the samples were kept cool to avoid carotenoids degradation. Carotenoid detection was performed on an Acquity UPC^2 (Waters Corporation, Milford, MA, USA) and automatic import of spectra and data acquisition were performed with MassLynx software (Waters Corporation, Milford, MA, USA). The list of the carotenoids analyzed for each stage is shown in Table 3.

Table 3: list of carotenoids extracted at the breaker and ripe stages.

Carotenoid	Code	Breaker stage	Ripe stage
Lycopene	LYC	x	x
β-carotene	β-CAR	x	x
Phytoene	PHY	x	x
Phytofluene	PFLU	x	x

1.2.5 Statistical analysis

Unless specified, all the statistical analyses were performed by using JMP 7 (SAS Institute, Inc.).

For the phenotypic and carotenoids content studies, collected data were analyzed by one-way analysis of variance (ANOVA) to test the presence of significant differences among genotypes or between groups (Landraces and Commercial varieties). In this latest case, the vintage variety “Varrone” was included in the group of the landraces. When necessary, means were separated according to the Tukey honest significant difference (HSD) at $p<0.05$ level. Graphics were created using MS-Excel 2016.

The interaction analysis between the two experimental greenhouse trials was performed considering only the accessions shared among the two.

Nei's diversity index (Nei, 1978) was used in order to evaluate the diversity among morpho-phenological qualitative traits, calculated by GenAlEx software (Genetic Analysis in Excel) (Peakall e Smouse, 2012).

Broad sense heritability was calculated by fitting the model with random effects through the restricted maximum likelihood method (REML) (Piepho e Möring, 2007).

Pearson's correlations were estimated to verify associations among different traits.

Multivariate analyses were used to obtain an overview of the data variability by Principal Component Analysis (PCA). The principal components (PCs) with eigenvalues ≥1 were retained for data discussion (Dunteman, 1989) and the correlations between each parameter and the PCs were calculated. The results of the PCA are shown as biplots of scores and loadings, drawn by using ggbiplot package of R CRAN network (http://github.com/vqv/ggbiplot).

Descriptive genetic statistics were calculated for the overall collection and within each group of genotypes by using GenAlEx software (Genetic Analysis in Excel) (Peakall e Smouse, 2012). The genetic distances among the accession were determined using TASSEL 2.1 software (Bradbury et al., 2007). TASSEL calculate the distances as 1-IBS (identity by state) similarity, with IBS defined as the probability that alleles drawn at random from two individuals at the same locus are the same. Genetic relationships among the accessions were determined by using MEGA X software (Kumar et al., 2018). A phylogenetic tree was constructed by the Neighbor-Joining method based on the genetic distances among accessions. For clustering, the distance of an individual from itself is set to 0.

For the metabolic study, statistical, multivariate and clustering analyses were performed using MetaboAnalyst 4.0 open source web application software (https://www.metaboanalyst.ca/) (Chong et al., 2019). Principal component analysis (PCA) was used to obtain an overview of the variability in

the metabolite profiles among the accessions. Partial Last Square Discriminant Analysis (PLS-DA) was implemented to maximize the separation between groups. Discriminant metabolites were selected from the VIP-plot retaining VIP>1. The quality of the model was validated on the basis of R^2 and Q^2 parameters in cross-validation. Cluster analysis was performed by hierarchical method visualizing the data through heatmaps.

1.3 Results

1.3.1 Phenotypic analysis

Preliminary analyzes based on the results obtained during the EX1, allowed to determine the variability among accession for all the evaluated traits and set the criteria to identify the landraces less adapted to greenhouse conditions or that responded poorly to the management, and, consequently, to choose the varieties to be included in the next experimental trial. Two characteristics of greatest interest for a farmer were considered: the flowering-ripening interval (*FRI*) and the average weight of the fruit (*FWG*). As a result, late-ripening varieties, i.e. with a flowering-ripening interval (*FRI*) greater than 80 days, were excluded. These varieties were also those with a fruit of considerable size, i.e. with an average weight of the fruit (*FWG*) greater than 300 g (P08, P29, P30, P33). Varieties that showed anomalies in the inflorescence or displayed a determinate growth habit were also excluded (P02 and P52). Therefore, out of 12 varieties present in the EX1, six were excluded for the EX2 (Tab. 1). The following analyzes were performed considering only the accessions shared among the two experimental greenhouse trials.

The ANOVA analysis for all the quantitative traits evaluated in EX1 and EX2 was performed using the year, the genotype and the interaction year x

genotype as effects of the model (Tab. 4). The year is highly statistically significant for the days to flowering from sowing (*DTFs*), the days to flowering from transplanting (*DTFt*) and the number of flowers per inflorescence (*NFI*) (Tab. 4). Strong significant differences (P < 0.0001) among genotypes were detected for all the parameters (Tab. 4). For the interaction year x genotype strong significant differences (P < 0.0001) for all the quantitative traits were detected, except for the flowering-ripening interval (*FRI*), the fruit weight (*FWG*), and the number of locules (*NOL*) (Tab. 4).

Table 4: ANOVA analysis for all quantitative traits evaluated in EX1 and EX2. Year, genotype and the interaction year × genotype have been considered as effects of the model.

Trait[a]	Year				Genotype				Year X Genotype			
	DF	SS	F		DF	SS	F		DF	SS	F	
DTFs	1	1379.86	50.32	****	9	9297.80	37.67	****	9	1069.30	4.33	****
DTFt	1	1491.48	57.90	****	9	8851.73	38.18	****	9	1552.45	6.70	****
FRI	1	293.18	2.88	n.s.	9	20118.01	21.99	****	9	1409.44	1.54	n.s.
NFI	1	1003.76	20.12	****	9	17387.10	38.73	****	9	3168.30	7.06	****
FWG	1	9162.60	5.12	*	9	2549642.10	158.46	****	9	30514.00	1.90	n.s.
FLE	1	0.06	0.26	n.s.	9	425.22	203.50	****	9	19.15	9.16	****
FWI	1	1.46	3.19	n.s.	9	1135.19	275.43	****	9	13.04	3.16	**
FL/W	1	0.13	13.57	***	9	31.54	377.93	****	9	1.90	22.81	****
NOL	1	0.29	0.10	n.s.	9	3977.62	158.43	****	9	34.47	1.37	n.s.

* P< 0,05; ** P< 0,01; *** P<0,001; **** P< 0,0001; n.s: not significant

[a] DTFs = days to flowering from sowing; DTFt = days to flowering from transplanting); FRI = flowering-ripening interval (days); NFI = number of flowers per inflorescence; FWG = mean fruit weight (g); FLE = fruit length (cm); FWI = fruit width (cm); FL/W = fruit length/width; NOL = number of locules.

Mean, maximum and minimum values of the quantitative traits showed a wide variation in both trials (Tab. 5). This variation is highly significant (P < 0.0001) for all the traits both in EX1 and in EX2. This was particular evident for the number of flowers per inflorescence (*NFI*), the fruit weight (*FWG*) and the number of locules (*NOL*), showing high coefficient of variation (*CV*) of 66%, 66% and 76%, respectively, in EX1 and 90%, 62% and 63%, respectively, in EX2.

The Nei's diversity index (*He*) calculated among qualitative traits ranged from 0.51 for the fruit cross-sectional shape (*FSS*) to 0.76 for the fruit shape (*FSH*) in EX1 and from 0.20 for the fruit cross-sectional shape (*FSS*) to 0.89 for the fruit shape (*FSH*) in EX2 (Tab. 6). All traits showed the same number of variants in the two trials only for the inflorescence type (*ITP*) and the shape of pistil scare (*SPS*), whereas for fruit shape (*FSH*) and fruit color (*FCO*) one more variant was detected in EX2, for fruit blossom and shape (*SBE*) and fruit cross-sectional shape (*FSS*) one lesser variant was detected in EX2 (Tab. 6).

Table 5: Differences among accessions for all quantitative traits in EX1 and EX2

EX1								
Trait[a]	Max	Min	Media	SD	DF	SS	F	P
DTFs	85	48	61	7.22	9	2913.03	11.91	****
DTFt	51	20	32	6.18	9	2118.58	11.70	****
FRI	102	36	69	13.56	9	6051.99	4.84	****
NFI	36	2	10	6.62	9	2383.26	11.54	****
FWG	454.1	12.2	154.88	101.80	9	914652.80	45.57	****
FLE	9.2	3.9	5.99	1.08	9	107.55	56.82	****
FWI	12.2	2	6.52	2.11	9	424.87	74.78	****
FL/W	2.3	0.6	1.03	0.39	9	15.97	179.26	****
NOL	21	2	6	4.37	9	1586.76	34.94	****
EX2								
Trait[a]	Max	Min	Media	SD	DF	SS	F	P
DTFs	99	49	66	8.17	9	9610.84	38.80	****
DTFt	71	19	37	8.65	9	11372.98	44.85	****
FRI	110	37	66	12.97	9	20192.25	26.24	****
NFI	64	1	14	13.09	9	26289.01	49.57	****
FWG	464.5	12.1	164.08	101.00	9	2071633.70	144.45	****
FLE	10.7	3.6	5.97	1.47	9	460.09	211.46	****
FWI	10.6	2.3	6.66	2.02	9	883.73	257.16	****
FL/W	1.9	0.6	0.97	0.29	9	18.61	229.91	****
NOL	17	2	6	3.69	9	2840.10	175.40	****

* P< 0,05; ** P< 0,01; *** P<0,001; **** P< 0,0001; n.s: not significant
Min=minimum value, Max= maximum value, SD= standard deviation, DF= degrees of freedom, SS= sum of squares, F= F ratio
[a] DTFs = days to flowering from sowing; DTFt = days to flowering from transplanting; FRI = flowering-ripening interval; NFI = number of flowers per inflorescence; FWG = mean fruit weight (g); FLE = fruit length (cm); FWI = fruit width (cm); FL/W = fruit length/width; NOL = number of locules.

Broad sense heritability (H^2) for quantitative traits showed an average value of 70% and varied between 42% for the flowering-ripening interval (*FRI*) and 89% for the fruit length/width ratio (*FL/W*) (Fig. 2). Interestingly, the heritability for fruit morphology traits showed the highest values among all the analyzed traits (Fig. 2).

Table 6: Nei's diversity among genotypes for all qualitative traits in EX1 and EX2

Trait[a]	EX1				EX2			
	N[b]	Nc[c]	Ne[d]	He[e]	N[b]	Nc[c]	Ne[d]	He[e]
ITP	10	2	1.92	0.53	10	2	2.00	0.56
FSH	10	5	3.13	0.76	10	6	5.00	0.89
FCO	10	2	1.92	0.53	10	3	1.85	0.51
SBE	10	3	2.94	0.73	10	2	1.92	0.53
SPS	10	4	2.94	0.73	10	4	2.78	0.71
FSS	10	3	1.85	0.51	10	2	1.22	0.20
Mean				**0.63**				**0.57**

[a] ITP = inflorescence type; FSH = fruit shape; FCO = fruit color; SPS = shape of pistil scare; SBE = fruit blossom end shape; FSS = fruit cross-sectional shape;
[b] Number of observations, [c] Number of categories, [d] Number of effective categories, [e] Unbiased Nei's diversity (Nei, 1978).

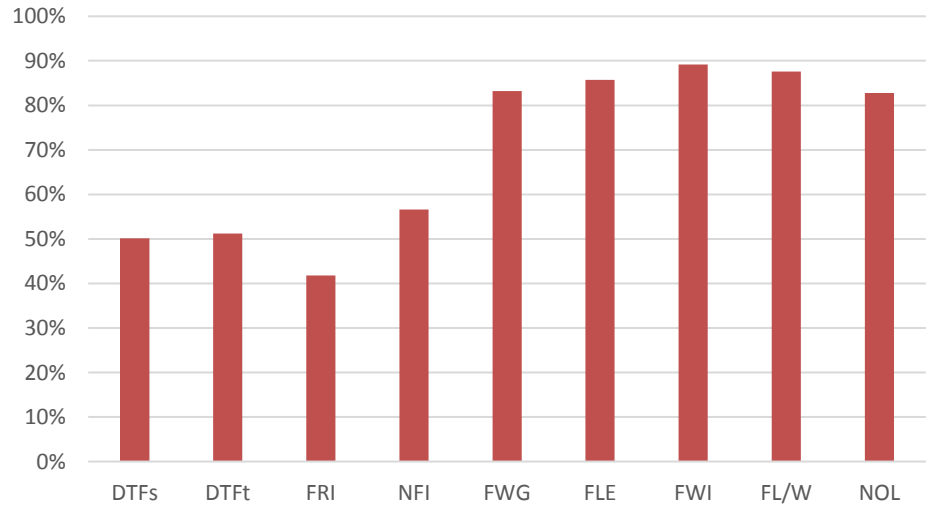


Figure 2: Broad sense heritability (H^2) among cultivated tomato for each quantitative trait evaluated in EX1 and EX2.

Note: DTFs = days to flowering from sowing; DTFt = days to flowering from transplanting; FRI = flowering-ripening interval (days); NFI = number of flowers per inflorescence; FWG = mean fruit weight (g); FLE = fruit length (cm); FWI = fruit width (cm); FL/W = fruit length/width; NOL = number of locules.

Pearson's correlations were calculated among quantitative traits in EX1 and EX2 (Tab. 7). Some significant correlations were detected among traits, such as the fruit weight (*FWG*), the fruit size (*FLE* and *FWI*) and the number of locules (*NOL*) for both trials. Also, the flowering-ripening interval (*FRI*) was positively correlated to the fruit weight (*FWG*), the fruit width (*FWI*), the fruit length/width (*FL*/*W*) and the number of locules (*NOL*) in both EX1 and EX2 (Tab. 7).

Table 7: Estimates of Pearson's correlations among accessions for all quantitative traits evaluated in EX1 and EX2.

EX1																			
Trait[a]	DTFs		DTFt		FRI		NFI		FWG		FLE		FWI		FL/W		NOL		
DTFs	-																		
DTFt	0.98	****	-																
FRI	0.81	**	0.86	**	-														
NFI	-0.45	n.s.	-0.50	n.s.	-0.35	n.s.	-												
FWG	0.37	n.s.	0.47	n.s.	0.71	*	-0.05	n.s.	-										
FLE	0.41	n.s.	0.46	n.s.	0.63	n.s.	0.00	n.s.	0.82	**	-								
FWI	0.52	n.s.	0.63	n.s.	0.78	**	-0.23	n.s.	0.95	****	0.71	n.s.	-						
FL/W	-0.58	n.s.	-0.68	*	-0.67	*	0.56	n.s.	-0.61	n.s.	-0.27	n.s.	-0.82	n.s.	-				
NOL	0.19	n.s.	0.26	n.s.	0.43	n.s.	0.30	n.s.	0.84	**	0.70	*	0.78	**	-0.46	n.s.	-		
EX2																			
Trait[a]	DTFs		DTFt		FRI		NFI		FWG		FLE		FWI		FL/W		NOL		
DTFs	-																		
DTFt	0.99	****	-																
FRI	0.87	**	0.87	**	-														
NFI	-0.18	n.s.	-0.16	n.s.	-0.47		-												
FWG	0.55	n.s.	0.55	n.s.	0.65	*	0.05	n.s.	-										
FLE	0.55	n.s.	0.56	n.s.	0.60	n.s.	0.05	n.s.	0.80	**	-								
FWI	0.63	n.s.	0.63	n.s.	0.76	*	-0.13	n.s.	0.95	****	0.67	*	-						
FL/W	-0.47	n.s.	-0.46	n.s.	-0.64	*	0.43	n.s.	-0.54	n.s.	-0.10	n.s.	-0.77	**	-				
NOL	0.42	n.s.	0.45	n.s.	0.41	n.s.	0.36	n.s.	0.85	**	0.72	*	0.78	**	-0.38	n.s.	-		

* P< 0,05; ** P< 0,01; *** P<0,001; **** P< 0,0001; n.s: not significant

[a] DTFs = days to flowering from sowing; DTFt = days to flowering from transplanting ; FRI = flowering-ripening interval (days); NFI = number of flowers per inflorescence; FWG = mean fruit weight (g); FLE = fruit length (cm); FWI = fruit width (cm); FL/W = fruit length/width; NOL = number of locules.

Relations among quantitative and qualitative traits in EX1 and EX2 were also observed (Tab. 8). Some correlations were found in EX1: as an example, the number of flowers per inflorescence (*NFI*) was positively correlated to the inflorescence type (*ITP*), and the number of locules (*NOL*) was positively correlated to the fruit shape (*FSH*) in both EX1 and EX2 (Tab. 8).

Table 8: ANOVA analysis and R^2 values between quantitative and qualitative traits evaluated in EX1 and EX2 among accessions.

EX1												
Trait[a]	ITP		FSH		FCO		SBE		SPS		FSS	
DTFs	0.03	n.s.	0.14	n.s.	0.32	n.s.	0.09	n.s.	0.39	n.s.	0.09	n.s.
DTFt	0.05	n.s.	0.22	n.s.	0.24	n.s.	0.12	n.s.	0.39	n.s.	0.04	n.s.
FRI	0.02	n.s.	0.30	n.s.	0.06	n.s.	0.16	n.s.	0.38	n.s.	0.08	n.s.
NFI	0.48	*	0.43	n.s.	0.10	n.s.	0.38	n.s.	0.37	n.s.	0.19	n.s.
FWG	0.06	n.s.	0.79	n.s.	0.31	n.s.	0.20	n.s.	0.47	n.s.	0.59	*
FLE	0.00	n.s.	0.64	n.s.	0.17	n.s.	0.15	n.s.	0.19	n.s.	0.72	*
FWI	0.03	n.s.	0.78	n.s.	0.34	n.s.	0.19	n.s.	0.64	n.s.	0.40	n.s.
FL/W	0.00	n.s.	0.76	n.s.	0.22	n.s.	0.40	n.s.	0.68	n.s.	0.07	n.s.
NOL	0.38	n.s.	0.90	*	0.57	n.s.	0.01	n.s.	0.67	n.s.	0.82	n.s.
EX2												
Trait[a]	ITP		FSH		FCO		SBE		SPS		FSS	
DTFs	0.03	n.s.	0.40	n.s.	0.58	n.s.	0.07	n.s.	0.60	n.s.	0.08	n.s.
DTFt	0.04	n.s.	0.40	n.s.	0.65	n.s.	0.09	n.s.	0.64	n.s.	0.06	n.s.
FRI	0.00	n.s.	0.52	n.s.	0.33	n.s.	0.00	n.s.	0.81	*	0.05	n.s.
NFI	0.42	*	0.70	n.s.	0.31	n.s.	0.21	n.s.	0.54	n.s.	0.02	n.s.
FWG	0.20	n.s.	0.74	n.s.	0.54	n.s.	0.03	n.s.	0.52	n.s.	0.06	n.s.
FLE	0.15	n.s.	0.73	n.s.	0.70	n.s.	0.00	n.s.	0.34	n.s.	0.16	n.s.
FWI	0.06	n.s.	0.77	n.s.	0.40	n.s.	0.05	n.s.	0.73	*	0.00	n.s.
FL/W	0.04	n.s.	0.87	n.s.	0.12	n.s.	0.17	n.s.	0.69	n.s.	0.11	n.s.
NOL	0.37	n.s.	0.91	*	0.77	*	0.02	n.s.	0.70	n.s.	0.00	n.s.

[a] DTFs = days to flowering from sowing; DTFt = days to flowering from transplanting; FRI = flowering-ripening interval (days); NFI = number of flowers per inflorescence; FWG = mean fruit weight (g); FLE = fruit length (cm); FWI = fruit width (cm); FL/W = fruit length/width; NOL = number of locules; ITP = inflorescence type; FSH = fruit shape; FCO = fruit color; SPS = shape of pistil scare; SBE = fruit blossom end shape; FSS = fruit cross-sectional shape.

Principal component analysis (PCA) for all the quantitative traits showed that the first three principal components (PCs) cumulatively explain nearly the 92% of the total phenotypic variation in both EX1 and EX2 (Tab. 9).

Table 9: Total variance explained by the first three PCs and their eigenvalues in EX1 and EX2 and among all the accessions.

EX1				EX2			
Component	**Total**	**% of variance**	**Cum %**	**Component**	**Total**	**% of variance**	**Cum %**
1	5.50	61.16	61.16	1	5.48	60.94	60.94
2	1.99	22.13	83.29	2	1.76	19.53	80.46
3	0.79	8.75	92.03	3	0.98	10.86	91.32

In detail, in EX1 the PC1 captured about the 61% of the total variation (Tab. 9) and showed loadings for all traits excepted for *NFI*, *ITP*, *FSH*, *SBE*, *FSS* and *FCO* (Tab. 10); the PC2 explained around 22% of the total phenotypic variation (Tab. 9) and was correlated to *NFI*, *NOL*, *FSS* and *ITP* (Tab. 10). The PC3 explained an additional 9% of the total variation (Tab. 9).

In EX2, the PC1 captured about the 61% of the total variation (Tab. 9) and showed high loadings for *DTFs*, *DTFt*, *FRI*, *FWG*, *FLE*, *FWI*, *FL/W*, *NOL*, *SPS* and *FCO* (Tab. 10); the PC2 explained around 19% of the total phenotypic variation (Tab. 9) and was correlated to *NFI*, *NOL*, *ITP* and *FSH* (Tab. 10). The PC3 explained an additional 11% of the total variation (Tab. 9).

The first two principal components, as calculated for both trials, are plotted in Figure 3. Regarding the EX1, the PCA allowed to separate the collection into different groups: the landraces P46 and P36 plotted together and present negative values for both the PC2 and PC1 (Fig. 3 A). These two landraces were characterized by bigger, heart or pear-shaped fruits and high number of flowers per inflorescence. The commercial variety C3, the smallest one with the earlier ripening date, was distinctly differentiate from the other accessions, with positive values of the PC1 and negative values of the PC2 (Fig.

3 A). The other varieties presented positive values for the PC2 and were separated by the PC1: the P113, P05, P01 landraces and the commercial variety C1 plotted together with positive values of PC1 and were characterized by medium-small, elongated and rounded fruits and a shorter flowering-ripening interval; while the P16 and P04 landraces and the commercial variety C2 had negative values of PC1 and were characterized by bigger and flat fruits with a higher flowering-ripening interval (Fig. 3 A).

Table 10: Correlation between the first three PCs and all phenotypic traits among all accessions.

Trait[a]	EX1			EX2		
	Component			Component		
	1	2	3	1	2	3
DTFs	0.76	-0.48	0.38	0.77	-0.34	0.52
DTFt	0.84	-0.45	0.27	0.80	-0.31	0.50
FRI	0.91	-0.16	0.21	0.87	-0.33	0.01
NFI	-0.36	0.77	0.35	-0.17	0.79	0.44
FWG	0.86	0.44	-0.12	0.89	0.35	-0.19
FLE	0.74	0.43	0.29	0.75	0.37	0.12
FWI	0.94	0.22	-0.24	0.94	0.12	-0.27
FL/W	-0.80	0.24	0.49	-0.72	0.32	0.40
NOL	0.67	0.68	-0.10	0.72	0.61	-0.14
ITP	0.01	0.67	-0.01	0.16	0.67	0.10
FSH	-0.01	0.51	0.17	0.00	0.48	0.20
FCO	-0.38	-0.55	0.44	-0.52	-0.44	0.11
SBE	-0.41	0.24	0.52	-0.27	0.18	0.40
SPS	0.64	0.22	-0.11	0.72	0.13	-0.04
FSS	0.47	0.67	0.11	0.17	0.26	-0.41

[a] DTFs = days to flowering from sowing; DTFt = days to flowering from transplanting; FRI = flowering-ripening interval (days); NFI = number of flowers per inflorescence; FWG = mean fruit weight (g); FLE = fruit length (cm); FWI = fruit width (cm); FL/W = fruit length/width; NOL = number of locules; ITP = inflorescence type; FSH = fruit shape; FCO = fruit color; SPS = shape of pistil scare; SBE = fruit blossom end shape; FSS = fruit cross-sectional shape.

PCA was also performed for the EX2 and showed a distribution of the varieties similar to that of the EX1: the commercial variety C3 is distinctly differentiated from the other accessions; the P46 and P36 landraces are outliers in respect to the main group (Fig. 3 C).

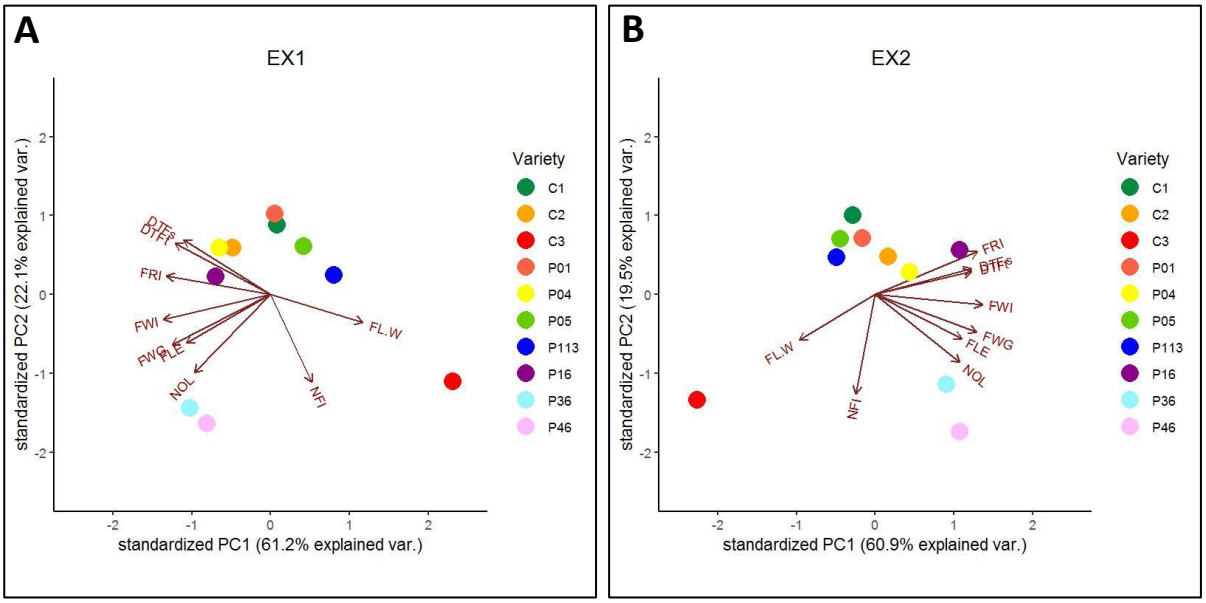


Figure 3: Scatter and loading plots of the first and second components obtained by the principal component analysis (PCA) based on all evaluated traits in EX1 (A) and EX2 (B).

Note: DTFs = days to flowering from sowing; DTFt = days to flowering from transplanting; FRI = flowering-ripening interval (days); NFI = number of flowers per inflorescence; FWG = mean fruit weight (g); FLE = fruit length (cm); FWI = fruit width (cm); FL/W = fruit length/width; NOL = number of locules.

1.3.2 Molecular analysis

All of the 2165 selected SNPs were used for the genetic analysis. In Table 11 are showed the results of the genetic diversity within the landraces and commercial varieties groups and for all analyzed accessions. The landraces group showed 38% of private alleles and the commercial varieties showed 76% of private alleles (Tab. 11). The expected heterozygosity (*He*) was higher for

the commercial varieties (0.31) and lower for the landraces (0.05). The entire collection showed an expected heterozygosity of 0.19 (Tab. 11).

Table 11: Genetic diversity based on 2165 SNPs. Analyses have been performed both within groups (Landrace and Commercial Varieties) and overall the accessions (Total).

Collection	Sample	Na[a]	Ne[b]	Private alleles	He[c]
Landraces	13	1.2	1.1	38%	0.05
Commercial Varieties	3	1.6	1.6	76%	0.31
Total	16	2.0	1.3		0.19

[a] Observed number of alleles, [b] Effective number of alleles, [c] Expected heterozygosity Nei (1978)

The genetic relationships among all the 16 accessions were determined. The phylogenetic tree, constructed from the genetic distances among accessions, is showed in Figure 4.

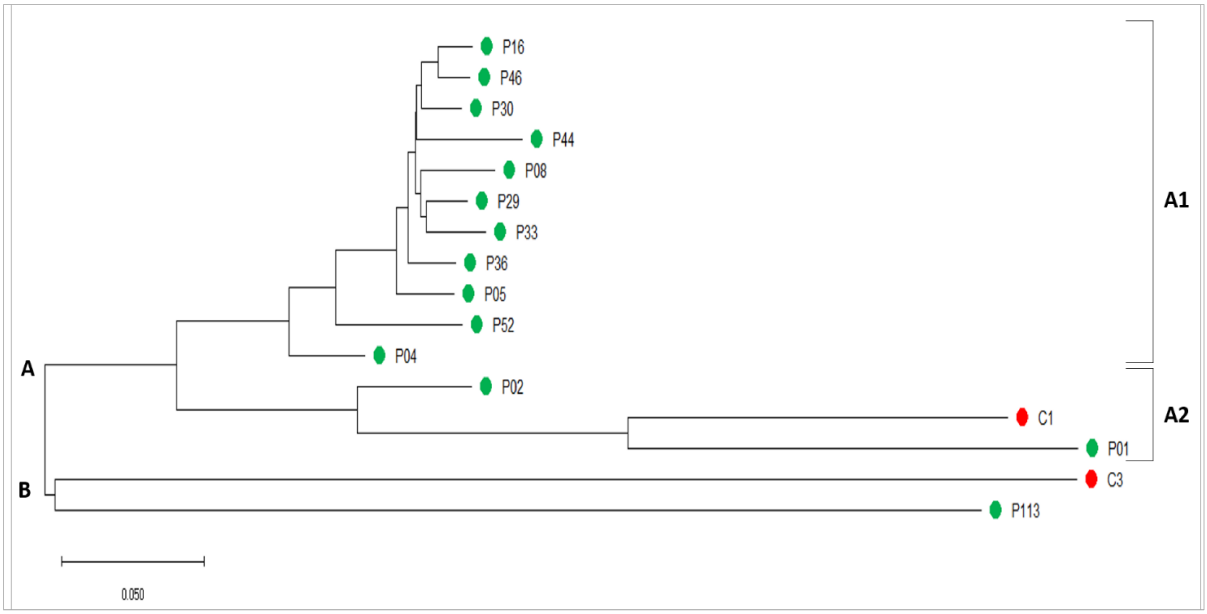


Figure 4: Phylogenetic tree of genetic relationships among all accession analyzed.

Note: Red dot = commercial variety; Green dot = landrace

In the dendrogram the landraces are colored in green and the commercial varieties in red. In the cluster analysis the accessions were split into two main groups (A and B). Group A was further subdivided into two

subgroups: A1 included only landraces, where two genotypes (P04 and P52) emerged as outlier; A2 was subdivided into two branches, the first including the landrace P02, the second including the landrace P01 and the commercial variety (C1), thus revealing a closer relationship between these two genotypes (Fig. 4). Group B included the landrace P113 and the commercial variety C3 thus resulting the most distant from the other genotypes (Fig. 4).

1.3.3 Metabolic analysis

The metabolic profile of the tomato accessions was represented with the aid of a heatmap and a cluster analysis. Heatmaps of the primary metabolites of all the accessions at the breaker and ripe stage is presented in Figure 5 A and B, respectively. These charts show the content of the metabolite in a color scale, where red is the highest concentration and blue the lowest. Metabolites content is plotted versus the accessions. The top dendrogram shows which accessions are the most similar based on the metabolites profile.

The heatmaps show a wide range of variation in the content of all primary metabolites in both breaker and ripe stage, but some accessions stood out for the content of specific metabolites (Fig. 5 A and B). For example, in the breaker stage the landrace P16 showed a reduce content of 17 primary metabolites, such as aspartic acid, threonic acid, leucine, isoleucine, glutamine, pyroglutamic acid and lysine, and an abundant content of other 4 primary metabolites, such as glyceric acid and fumaric acid (Fig. 5 A). On the contrary, an opposite profile of these metabolites was present in the landrace P44 (Fig. 5 A). The commercial variety C3 also showed an interesting primary metabolites profile, showing the highest content of 12 primary metabolites, such as maltose, sucrose, glucose-1-phosphate, glycerol, ascorbic acid and glucose (Fig. 5 A). Regarding the ripe group, the commercial variety C3 showed

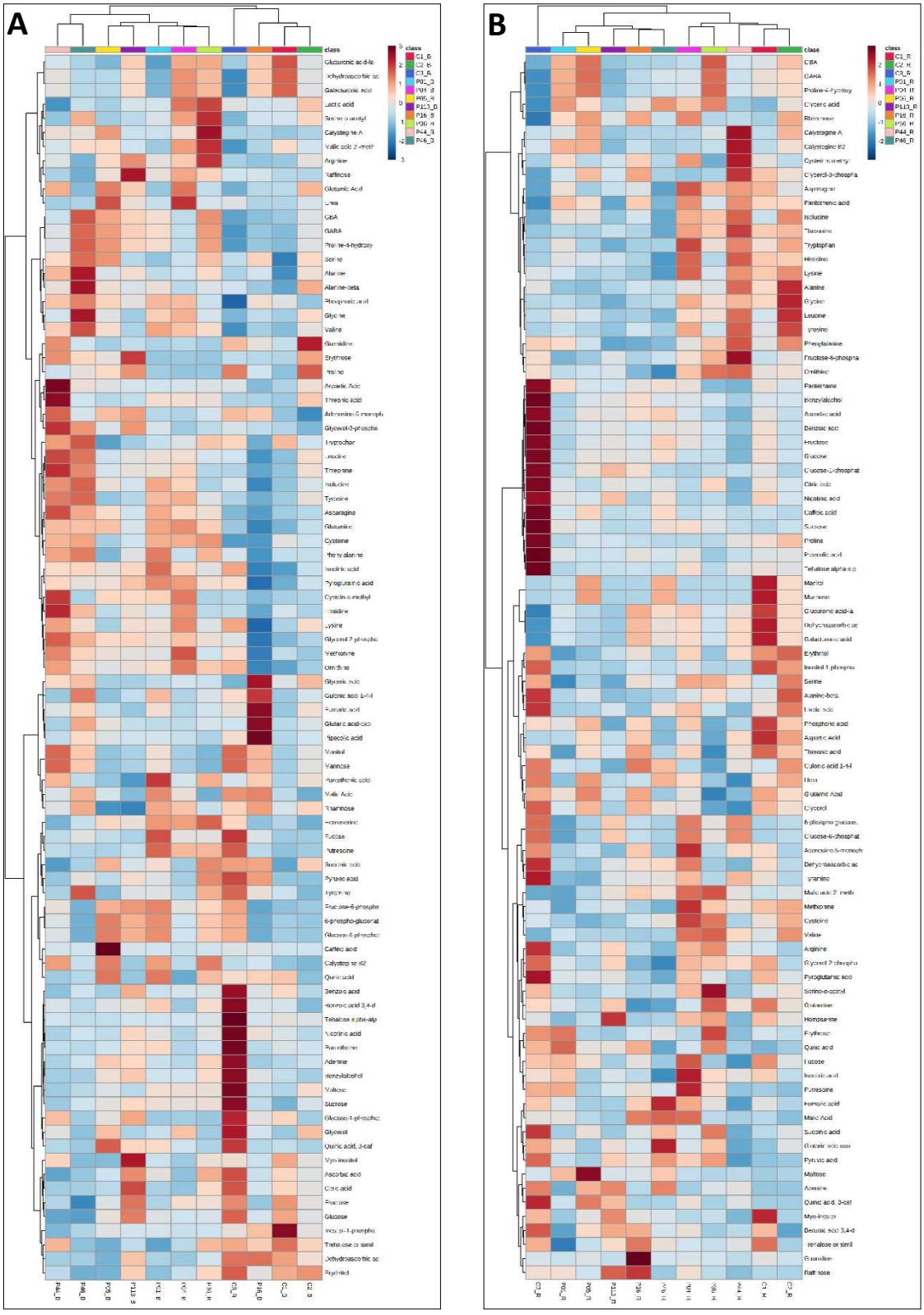


Figure 5: Heat Map of the primary metabolite profiles of all accessions in both breaker (A) and ripe stages (B).

again the highest content of 14 metabolites, such as ascorbic acid, fructose, glucose, citric acid and sucrose (Fig. 5).

To better evaluate the difference in primary metabolites content among all accessions and between landraces and commercial varieties, PCA and PLS-DA analysis were performed.

Principal component analysis (PCA) of all primary metabolites was performed to investigate the differences among all accessions in both breaker and ripe stage. The PCA showed that the first three principal components (PCs) cumulatively explain nearly the 63% of the total variation at breaker stage and the 60% of the total variation at the ripe stage (Tab. 12). In detail, at the breaker stage the PC1 captured about the 26% of the total variation, the PC2 explained around 25% of the total variation and the PC3 explained an additional 13% of the total variation (Tab. 12). At the ripe stage, the PC1 captured about the 29% of the total variation, the PC2 explained around 17% of the total variation and the PC3 explained an additional 13% of the total variation (Tab. 12).

Table 12: Total variance explained by the first three PCs among all the accessions at the breaker and ripe stage.

Breaker stage			Ripe stage		
Component	**% of variance**	**Cum %**	**Component**	**% of variance**	**Cum %**
1	25.80	25.80	1	29.30	29.30
2	24.80	50.60	2	17.40	46.80
3	12.80	63.50	3	13.50	60.30

The first two principal components are plotted in Figures 6 for both breaker and ripe stage. Regarding the breaker stage, the PCA allowed to distinctly separate two commercial varieties and two landraces from the main group of the other accessions (Fig. 6 A). In detail, the commercial variety C1

and the landraces P16 plotted together, with negative values for the PC1 and positive values for the PC2; the commercial variety C3 was clearly differentiated and presented negative values for both PC1 and PC2; the landrace P44 was plotted in the right bottom part of the PCA plot with positive values for the PC1 and negative values for the PC2 (Fig. 6 A).

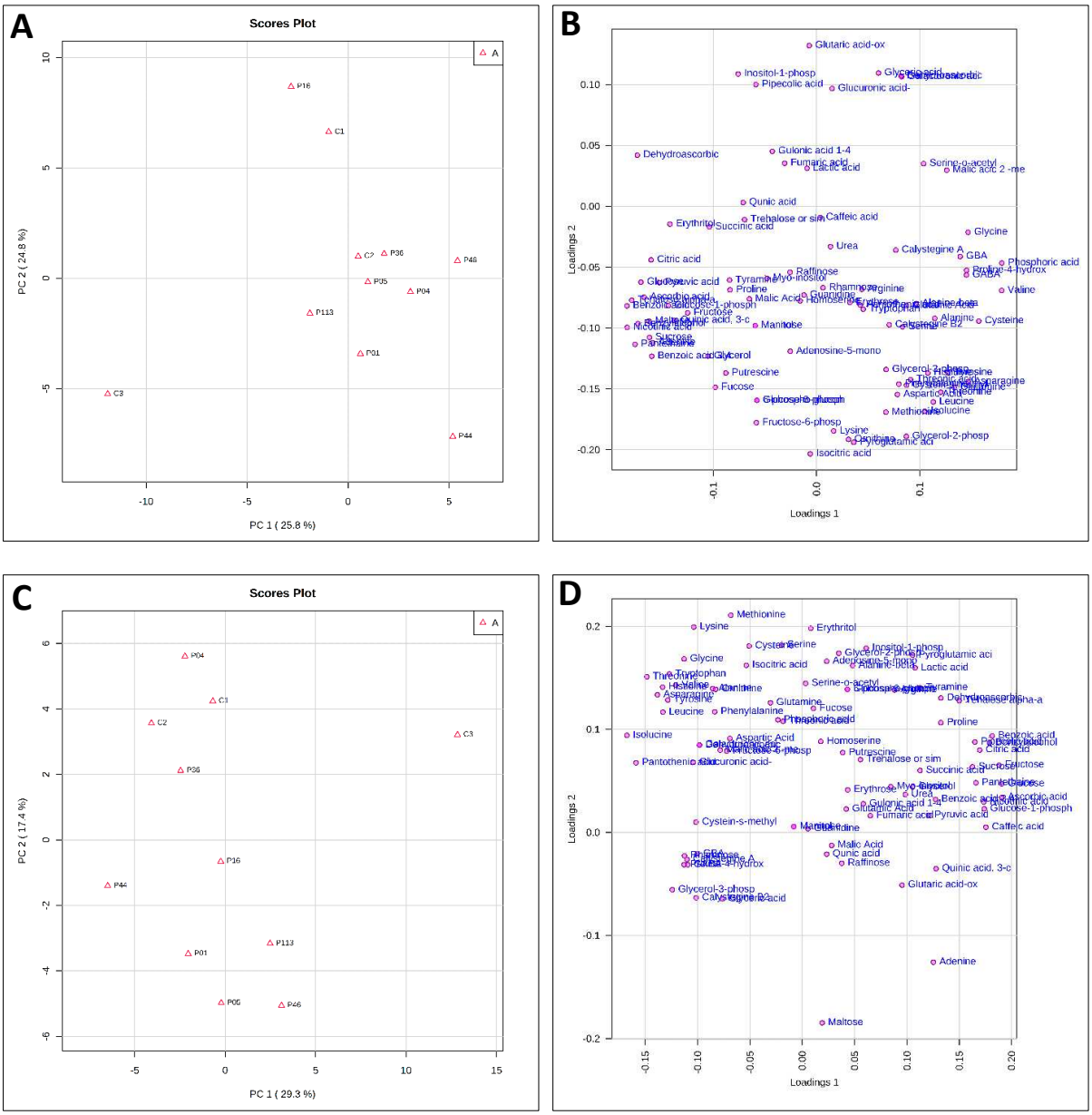


Figure 6: Scatter and loading plots of the first and second components obtained by the principal component analysis (PCA) based on all primary metabolites within both breaker (A and B) and ripe (C and D) stages.

At the ripe stage, the PCA allowed to separate the collection into different groups: the commercial variety C3 was again differentiated from all the other accessions; the commercial varieties C1 and C2 and the landraces P04 and P36 plotted together at the top of the PCA plot with negative values for the PC1 and positive value for the PC2; in an opposite position, the landraces P01, P05, P113, and P46 plotted together; the landraces P16 and P44 plotted between the two main groups, but clearly distant one from the other (Fig. 6 C). In Figures 6 B and D are represented the loadings of all primary metabolites for both breaker and ripe stages, respectively.

The PCA analysis did not allow a complete separation between the two groups of accessions. Hence, PLS-DA analysis was applied to better investigate the difference in the primary metabolites profile between the group of landraces and the group of commercial varieties, at both breaker and ripe stage. The PLS-DA showed that the first two components cumulatively explain nearly the 34% of the total variation at breaker stage and the 41% of the total variation at the ripe stage (Tab. 13). In detail, at the breaker stage, the first component captured about the 24% of the total variation and the second component explained around 10% of the total variation; whereas, at the ripe stage each of the first two components captured about the 21% of the total variation (Tab. 13). The variables (primary metabolites) with highest relevance in differentiating the samples were identified from VIP (Variable Importance in the Projection) values (VIP > 1) (Fig. 7).

Table 13: Total variance explained by the first two component between the landraces and commercial varieties groups at the breaker and ripe stage.

Breaker stage			Ripe stage		
Component	**% of variance**	**Cum %**	**Component**	**% of variance**	**Cum %**
1	23.90	23.90	1	20.70	20.70
2	9.80	33.70	2	21.10	41.80

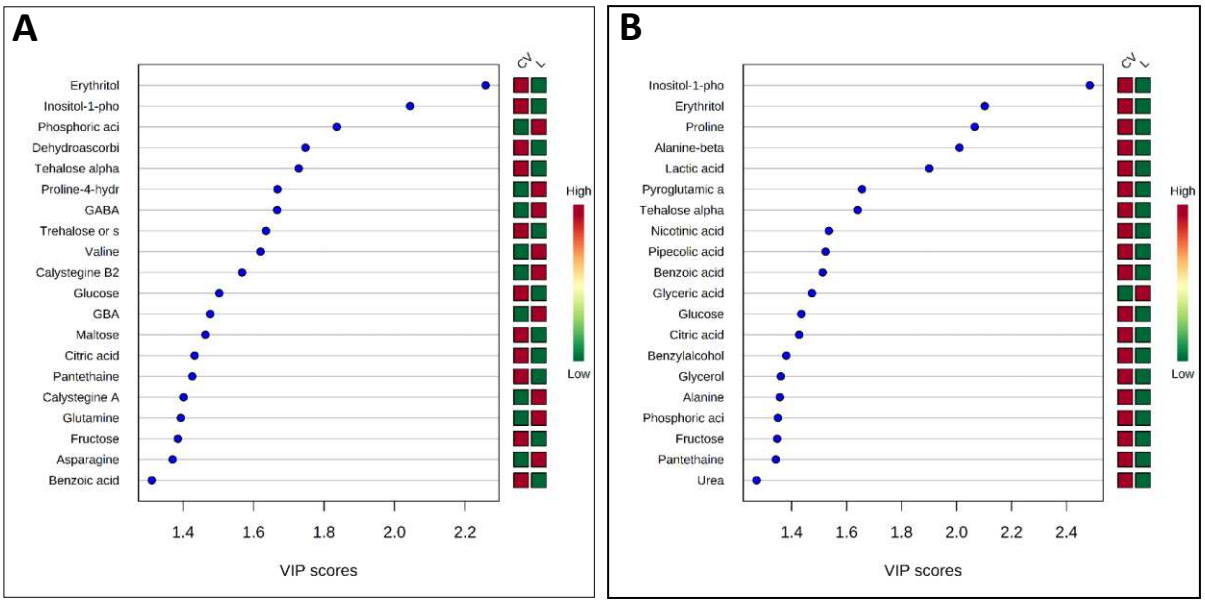


Figure 7: VIP (Variable Importance in Projection) score and relative concentrations of the corresponding metabolites in each group (on the right) for both breaker (A) and ripe stage (B).

Note: CV = commercial varieties; L = landraces

The first two components are plotted in Figures 8 for both breaker and ripe stage. The PLS-DA allowed a complete discrimination between the two groups in both stages: the group of the landraces was clearly separated from the group of commercial varieties by the first component (Fig. 8 A and C). Within the commercial variety group, the C3 is clearly separated by the second component from the C1 and C2 in both stages; within the landraces at the breaker stage the second component allowed to separate the accessions, highlighting the P16 and the P44 as substantially different, while, at the ripe stage the P44 is clearly separated from the other landraces. Among the key metabolites which provide the differentiation of the two groups, erythritol and inositol-1-phosfate were critical in both stages (Fig. 7 A and B). In Figures 8 B and D are represented the loadings of all primary metabolites in the differentiation of the two groups for both breaker and ripe stages, respectively.

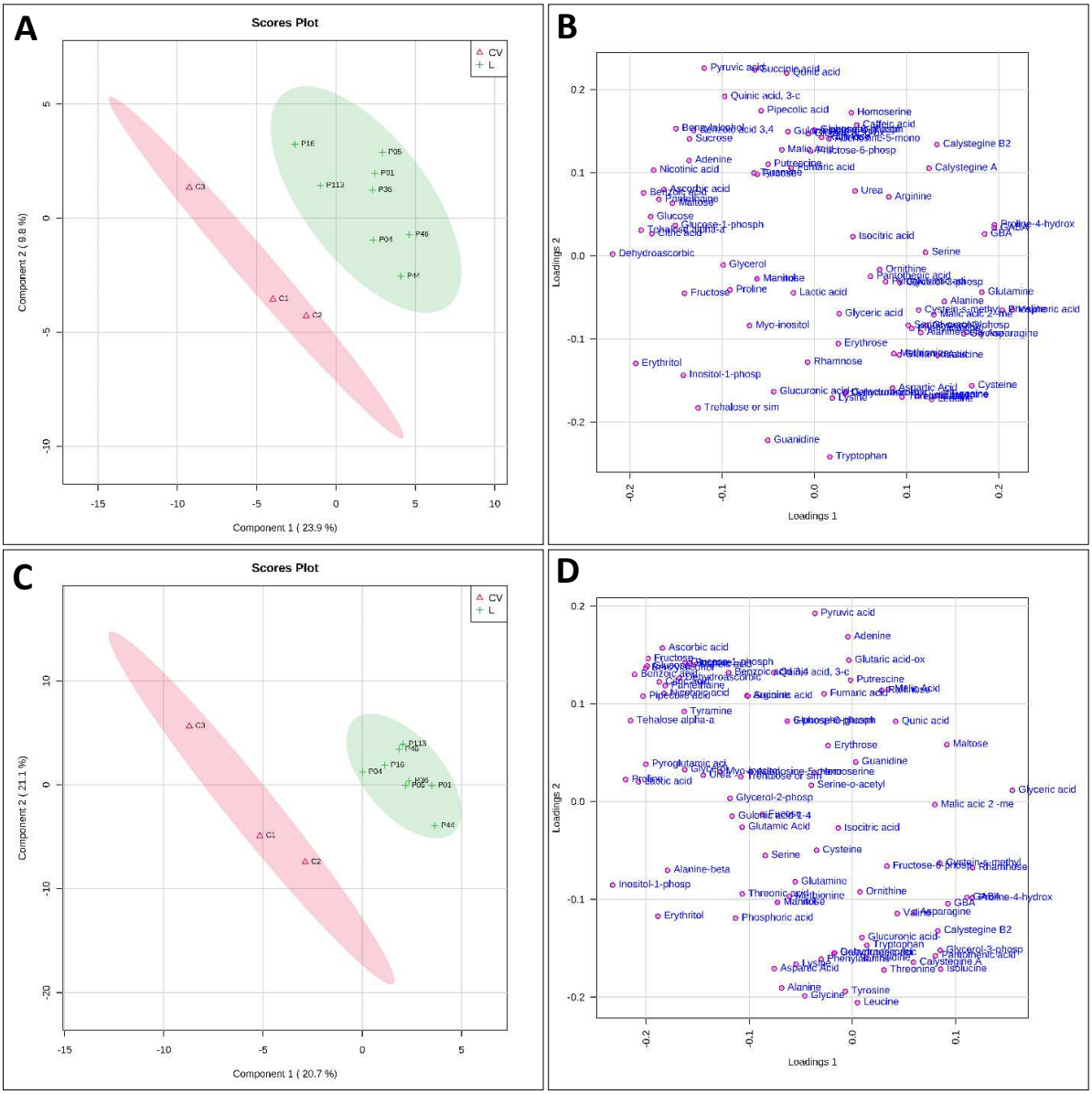


Figure 8: Scatter and loading plots of the first and second components obtained by the partial last square discriminant analysis (PLS-DA) based on all primary metabolites within both breaker (A and B) and ripe (C and D) stages.

Note: CV = commercial varieties; L = landraces

Among the secondary metabolites, a particular attention was deserved to carotenoids. As expected, the amount of carotenoids is higher at the ripe than at the breaker stage (Tab. 14). Indeed, no significant differences among genotypes were detected at the breaker stage, while all analyzed compounds show significant differences among genotypes at the red ripe stage (Tab. 14).

In detail, at the breaker stage the total mean carotenoids content was 6.36 mg/100 g dried sample weight (dsw) and increased of about 20-fold at the ripe stage (120.36 mg/100 g dsw) (Tab. 14). The lycopene (*LYC*) showed the highest increment, moving from a mean value of 0.42 mg/100 g dsw at the breaker stage to 64.28 mg/100 g dsw at the ripe stage. The phytoene (*PHY*), the second highest increment, increased its concentration moving from a mean value of 0.13 mg/100 g dsw to 22.84 mg/100 g dsw from the breaker stage to the ripe stage respectively (Tab. 14). The β-carotene (β-*CAR*) and the phytofluene (*PFLU*) showed the lowest increment, moving to 5.71 mg/100 g dsw and 0.10 mg/100 g dsw, respectively, at the breaker stage to 20.55 mg/100 g dsw and 12.69 mg/100 g dsw, respectively, at the ripe stage (Tab. 14).

Table 14: Significant differences among genotypes of cultivated tomato for all analyzed compounds.

Trait[a]	Stage[b]	Mean (mg/100 g dsw)	Max (mg/100 g dsw)	Min (mg/100 g dsw)	SD	DF	SS	F	P
PHY	B	0.13	1.79	0	0.43	10	1.60	0.79	n.s.
PFLU	B	0.10	1.49	0	0.33	10	0.89	0.82	n.s.
LYC	B	0.42	5.36	0	1.23	10	11.80	0.68	n.s.
β-CAR	B	5.71	21.88	2.50	3.78	10	129.95	0.91	n.s.
Total B stage		**6.36**							
PHY	R	22.84	125.00	6.25	22.75	10	12526.80	6.42	***
PFLU	R	12.69	70.00	3.49	12.62	10	3638.13	5.13	***
LYC	R	64.28	305.00	27.56	52.71	10	53147.81	3.05	*
β-CAR	R	20.55	95.00	7.67	15.81	10	5463.32	4.40	**
Total R stage		**120.36**							

* P< 0,05; ** P< 0,01; *** P<0,001; **** P< 0,0001; n.s: not significant
Min = minimum value, Max = maximum value, SD = standard deviation, DF = degrees of freedom, SS = sum of squares, F= F ratio
[a] PHY = phytoene; PFLU = phytofluene; LYC = lycopene; β-CAR = β-carotene
[b] B = breaker stage; R = ripe stage

The broad sense heritability (H^2) was calculated for each compound at the ripe stage only (Fig. 9), due to an irrelevant or very low carotenoids concentration at the breaker stage. Phytoene showed the highest values (H^2 = 66%), followed by phytofluene (H^2 = 60%), β-carotene (H^2 = 55%) and finally lycopene (H^2 = 42%). The mean H^2 showed an average value of 56%.

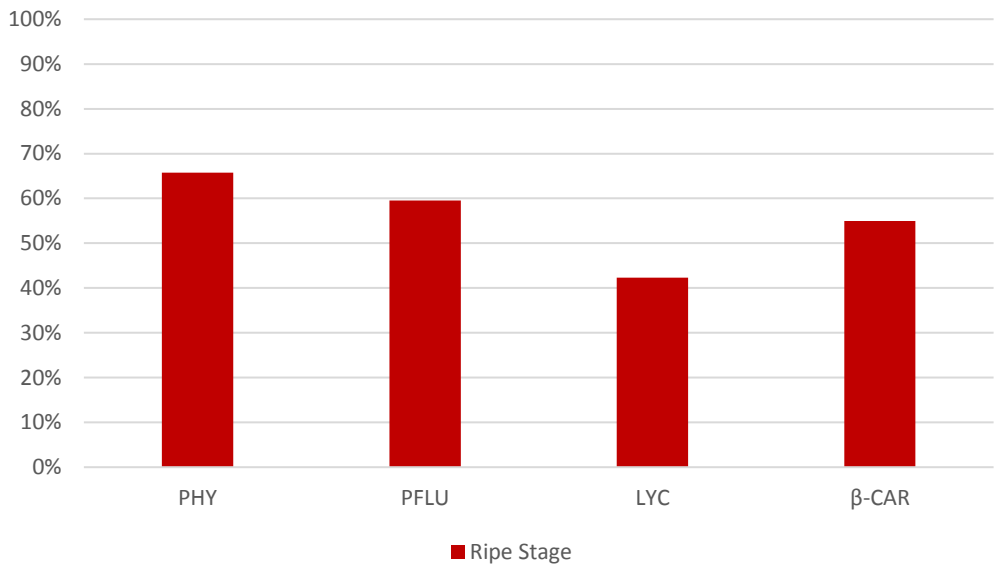


Figure 9: Broad sense heritability (H2) among accessions for each compound at ripe stage.
Note: PHY = phytoene; PFLU = phytofluene; LYC = lycopene; β-CAR = β-carotene

Pearson's correlations were observed among the different carotenoids content at both breaker and ripe stage (Tab. 15). At the breaker stage, the phytoene was positively correlated to the phytofluene and the lycopene, and the phytofluene was correlated to the lycopene content (Tab. 15). At the ripe stage, all carotenoids were highly positively correlated one to each other (Tab. 15). Significant correlations between the carotenoids content at the breaker stage and the carotenoids content at the ripe stage were not detected (Tab. 15). Also, correlation between phenotypic traits and carotenoids were investigated but no significant association was detected (data not shown).

Table 15: Estimates of Pearson's correlations among all the carotenoid contents at both breaker and ripe stage for all accessions.

Trait[a]	Breaker Stage								Ripe Stage							
	PHY		PFLU		LYC		β-CAR		PHY		PFLU		LYC		β-CAR	
Breaker Stage																
PHY	-															
PFLU	0.78	**	-													
LYC	0.86	***	0.68	*	-											
β-CAR	0.43	n.s.	0.23	n.s.	0.31	n.s.	-									
Ripe Stage																
PHY	-0.29	n.s.	-0.33	n.s.	-0.36	n.s.	-0.35	n.s.	-							
PFLU	-0.31	n.s.	-0.34	n.s.	-0.35	n.s.	-0.32	n.s.	1.00	****	-					
LYC	0.17	n.s.	0.12	n.s.	0.09	n.s.	0.12	n.s.	0.97	****	0.98	****	-			
β-CAR	-0.02	n.s.	-0.01	n.s.	-0.03	n.s.	-0.06	n.s.	0.97	****	0.97	****	0.97	****	-	

* P< 0,05; ** P< 0,01; *** P<0,001; **** P< 0,0001; n.s: not significant

[a] PHY = phytoene; PFLU = phytofluene; LYC= lycopene; β-CAR = β-carotene

Differences in carotenoids content between the groups of commercial varieties and landraces were investigated at both breaker and ripe stages (Fig. 10 A and B). In particular, at the breaker stage both commercial varieties and landraces showed zero or irrelevant concentration of phytoene, phytofluene and lycopene, while for the β-carotene the commercial varieties group showed the highest content (β-*CAR* = 6.87 mg/100 g dsw), though they were not significantly different from the landraces group (β-*CAR* = 5.27 mg/100 g dsw) (Fig. 10 A). At the ripe stage, the landraces group showed the highest content for all of the carotenoids, but no significant differences were detected with the commercial varieties group (Fig. 10 B). Among the four carotenoids observed, the lycopene showed the highest concentration in both commercial varieties (*LYC* = 57.10 mg/100 g dsw) and landraces (*LYC* = 66.97 mg/100 g dsw), but no significant differences were detected (Fig. 10 B).

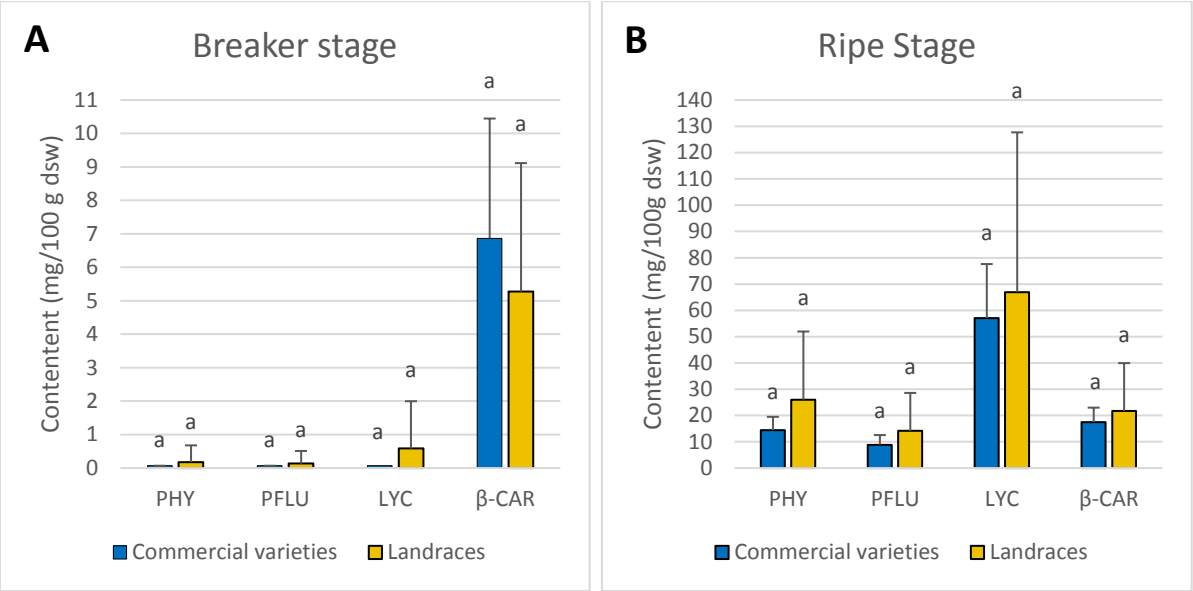
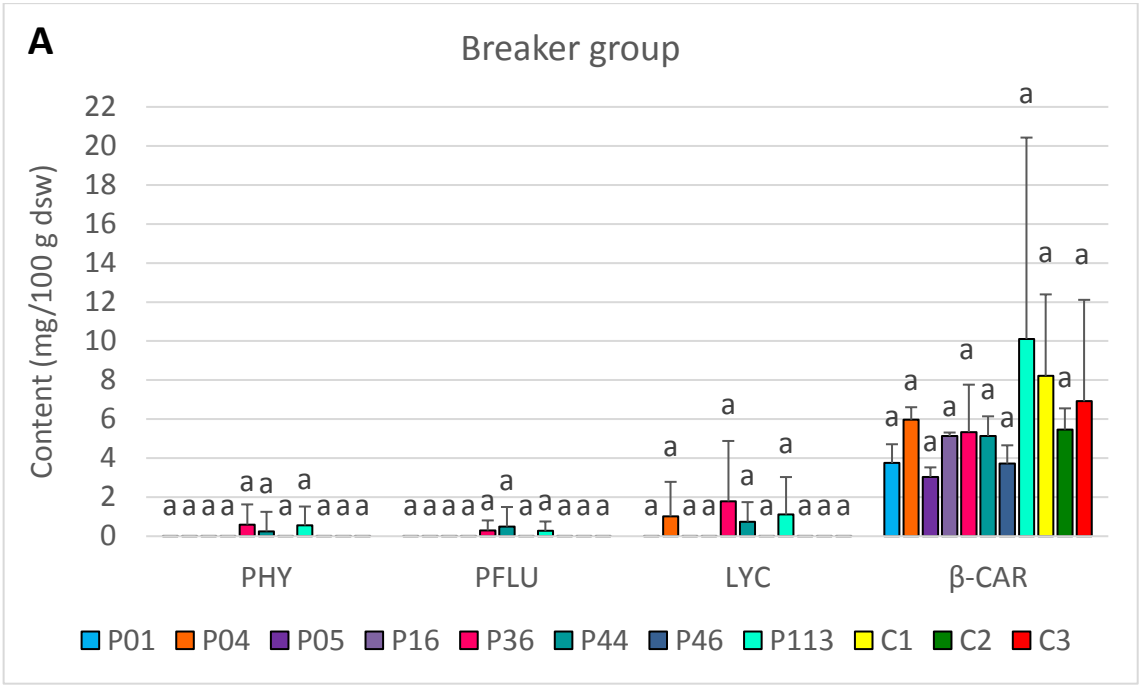


Figure 10: Differences in carotenoids contents between commercial varieties and landraces at the breaker (A) and ripe stage (B).

Note: PHY = phytoene; PFLU = phytofluene; LYC= lycopene; β-CAR = β-carotene
Means followed by different letters indicate significant differences among the groups based on Tukey-Kramer's test at $p < 0.05$. Vertical bars represent the standard deviation.

The differences in carotenoids content among all accessions were also investigated in both breaker and ripe stages (Fig. 11 A and B). In detail, at the breaker stage no significant differences were detected among genotypes (Fig. 11 A), whereas at the ripe stage, differences among accessions were significant for all of the compounds (Fig. 11 B). In particular, at the ripe stage the outstanding genotype for the phytoene, phytofluene and β-carotene contents was the landrace P04, Tamatticasa tundas a siccu, which showed 81.28 mg/100 g dsw, 43.59 mg/100 g dsw and 59.48 mg/100 g dsw values, respectively (Fig 11 B). No significant differences were detected among all other accessions (Fig. 11 B). For the lycopene content, the maximum value was registered for the accession P04 (180.26 mg/100 g dsw), followed by P46, P16 and C2 (79.59 mg/100 g dsw, 76.45 mg/100 g dsw and 74.44 mg/100 g dsw, respectively), which also showed no significant differences from the other accessions (Fig. 11 B).



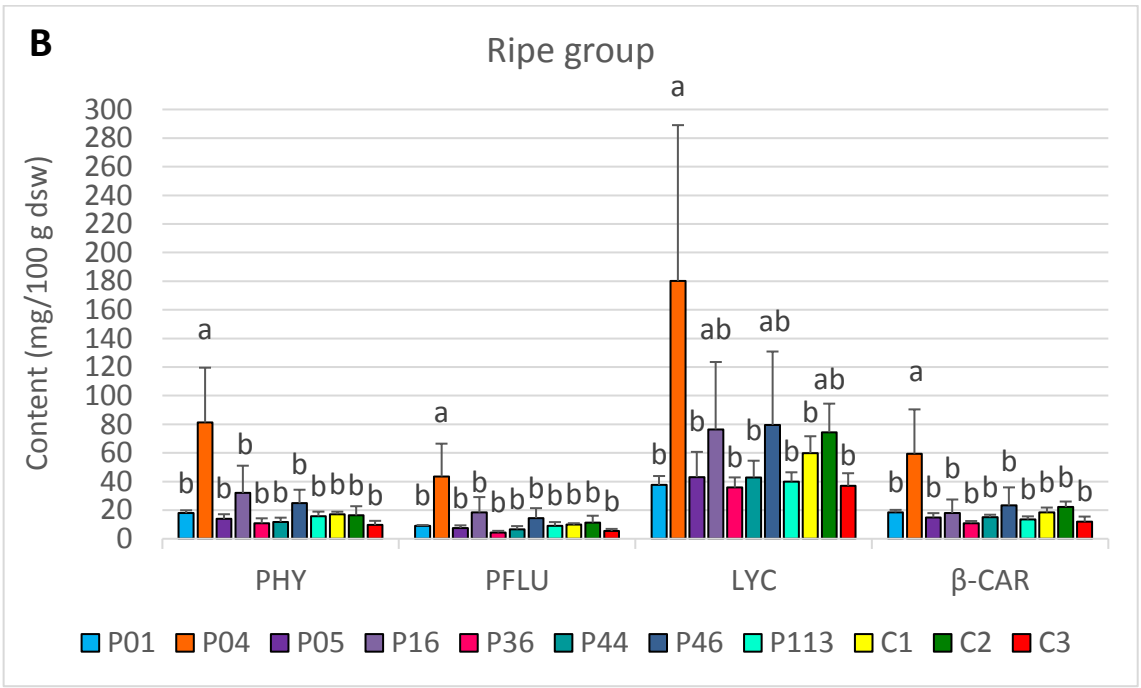


Figure 11: Carotenoids contents of all accession at the breaker (A) and ripe stage (B).

Note: PHY = phytoene; PFLU = phytofluene; LYC= lycopene; β-CAR = β-carotene
Means followed by different letters indicate significant differences among the varieties based on Tukey-Kramer's test at $p < 0.05$. Vertical bars represent the standard deviation.

1.4 Discussion

Differences in morpho-phenological, metabolic ad genetic composition were evaluated among the varieties object of this study, with the final aim to determine the suitability of the local tomato varieties to greenhouse conditions during the autumn/winter season under modern horticultural techniques and to enhance these local products promoting their direct use in local markets and valorizing their peculiarities in different contexts.

The whole collection evaluated during EX1 included one Italian vintage variety, three commercial varieties and 12 landraces with fruits of very variable shape and size. This range of variation is common in collections of local varieties (Panthee et al. 2013; Cebolla-Cornejo et al., 2013; Figàs et al., 2015a). Some of the variety evaluated in EX1 had a very large fruit size, with an average fruit weight (*FWG*) above 300 g and, consequently, a late-ripening stage (*FRI*), confirmed by their positive relation. These two characteristics are extremely important for a farmer who cultivates tomatoes in a greenhouse, especially in a season as the winter characterized by low temperatures and light, that can negatively influence the income of entrepreneur. In fact, its income is strictly linked to the availability and quantity of the product, whereby it will be higher as much as sooner the fruits will be ripe and ready to be placed on the market. For all these considerations, four of the 12 commercial varieties were excluded for the next experiment, since they showed characteristics that did not adapt well to greenhouse conditions during the autumn/winter season. As an example, among the typical Sardinian tomato excluded, "Tomattis mannu de Bachis", letteraly "Big tomato from Bachis", was characterized by flat shaped fruits with irregular cross-sectional shape, high fruit weight (up to 1000 g) and a mean flowering-ripening interval of 82 days. On the other side, in local markets the demand of fruits with these characteristics is increasing, as they are associated to intense flavor and high

quality by consumers, typical traits possessed by traditional local tomato varieties (Mazzucato et al., 2010; Figàs et al., 2015a; Lázaro et al., 2018). Also, other two varieties that showed anomalies in the inflorescence or displayed a determinate growth habit were excluded, as less adapted to the environment and the modern horticultural techniques used. However, all these six varieties can express their full potential, and thus obtain an adequate valorization, when cultivated in conditions more suitable to their characteristics, like open-field cultivation during the summer season or in the greenhouse during the spring season (Lázaro et al., 2018; Figàs et al., 2018).

Consequently, in EX2 six Sardinian local tomato varieties, one Italian vintage variety and three commercial varieties were included. The final collection, composed only by the accessions shared among the two experimental greenhouse trials, was characterized by a valuable level of phenotypic diversity, as already found in other collection of local tomato varieties (Mazzucato et al., 2008; Terzopoulos and Bebeli, 2010; Cebolla-Cornejo et al., 2013). Additionally, the high levels of heritability (H^2) observed for most of the registered traits indicate their elevated genetic control. In particular, among the traits related to the fruit morphology, the mean fruit weight (*FWG*) showed high heritability and high variation among accessions. Nei's diversity index (*He*) also revealed high values for some qualitative traits, such as for fruit shape (*FSH*). Fruit shape and fruit weigh are traits of major interest in fresh market cultivars and in breeding programmes (Foolad, 2007). Other studies on phenotypic diversity in tomato landraces from different countries, have shown similar or lower values of diversity (Terzopoulos and Bebeli, 2010; Figàs et al., 2018). Regarding the quantitative traits, for example, Figàs et al. (2018) evaluated and phenotypically characterized 12 tomato accessions from the region of València (Spain) in three different environments (open-field conventional, open-field organic and greenhouse), finding out heritability values for number of flower per inflorescence (*NFI*, H^2

= 0.56), fruit width (*FWI*, H^2 = 0.83) and fruit length (*FLE*, H^2 = 0.81) very close to those of the present study (0.57, 0.89, 0.86, respectively), for mean fruit weigh (*FWG*, H^2 = 0.73) slightly lower than the present study (H^2 = 0.83) and slightly higher for number of locules (*NOL*, H^2 = 0.92) than this research (H^2 = 0.83). Instead, concerning the qualitative traits, the diversity of the present collection was also interesting compared to that found by Terzopoulos and Bebeli (2010). They characterized and estimated the phenotypic diversity of 34 Greek tomato landraces using 36 morphological traits, finding lower or similar level of diversity than the present study for the traits fruit shape (*FSH*, *He* = 0.50), fruit color (*FCO*, *He* = 0.53), fruit blossom end shape (*SBE*, *He* = 0.48), shape of pistil scare (*SPS*, *He* = 0.64) and fruit cross-sectional shape (*FSS*, *He* = 0.60).

The principal component analyses performed on the basis of the morpho-phenological descriptors for both experiments, showed that some local varieties are clearly differentiated from the commercial varieties, while in some cases are generally intermixed. For example, in both EX1 and EX2 the commercial variety C3, the smallest one with the earlier ripening date, the landraces P36 and P46, characterized by bigger, heart or pear-shaped fruits, were clearly differentiated. This indicate that the morpho-phenological descriptor used allowed a clear and reliable distinction of the varieties, as found in other works (Cebolla-Cornejo et al., 2013; Figàs et al., 2015a; Figàs et al., 2018). Within the group including both landraces and commercial varieties having medium-small, elongated and rounded fruits and a shorter flowering-ripening interval, two interesting local varieties were found, the P01 and P04, named “Arracadas” and “Tamatticasa tundasa a siccu”, respectively. These varieties were selected by local farmers for different and peculiar traits: the “Arracadas”, literally “Earrings”, was mainly cultivated as long storage shelf-life variety (dried tomato) and “Tamatticasa tundasa a siccu”, literally “Round tomato grown in low water conditions“, was mainly cultivated for its

ability to grow and develop medium/big fruits even in scarce availability of water.

The level of the genetic diversity of the present collection is quite low (*He* = 0.19). In detail, the Sardinian landraces showed a very low diversity value, while the commercial varieties showed a valuable diversity value (*He* = 0.05 and 0.31, respectively). Sacco et al. (2015) evaluated 123 landraces from different geographical areas with the aim to capture a wide diversity. They investigated the genetic diversity within the groups of varieties, founding, on the contrary, a diversity value for the group of Italian landraces almost double than that found in the present study and for the cultivar group quite lower than that found in this research (*He* = 0.0951 and 0.1595, respectively). This difference is due to the very different number of accessions studied by Sacco et al (2015) than those evaluated in the present study (a total of 13 landraces). The highest number of private alleles was found for the commercial varieties group (76%), while only 38% of private alleles were detected among the local varieties. The cluster analysis based on genetic distances, showed that the collection is mainly divided into four genetic groups and reveled some interesting relationships among the accessions: the vintage variety “Varrone” and the commercial variety “Datterino” (P113 and C3, respectively) emerged as outliers and they were the most genetically distant from the other accessions; while, the landrace “Arracadas” (P01) and the commercial variety “Camone” (C1) revealed a closer relationship in respect to the remaining accessions. Interestingly, among the main group, only constituted to landraces, the landrace P04, the aforementioned "Tamatticasa tundas a siccu", again stood out as particularly different. However, as expected, the differences of accessions in fruit morphology did not completely match with the phylogenetic clustering, as before demonstrated in different tomato collections (Mazzucato et al., 2008; Sacco et al., 2015).

The primary metabolite analysis provided a powerful and reliable approach to study changes in the metabolite level of landraces. The PLS-DA analysis showed a clear separation between the landraces and the commercial varieties groups in both ripening stages (breaker and ripe), demonstrating that the landraces are a valuable material for metabolite profile studies (Baldina et al., 2016, Zhu et al., 2018). Additionally, the PCA allowed to identify some interesting varieties, different form the others for some important metabolites. In detail, two landraces and one commercial variety stand out in both breaker and ripe stage: the landraces P16 and P44 and the commercial variety C3. These accessions showed a different metabolite profile at both breaker and ripe stage, especially for some interesting metabolite related to tomato flavors, primarily defined by sugars such as glucose, sucrose and fructose, and by volatile compounds as leucine (Thoge and Fernie, 2015). For example, comparing these three varieties, the landrace P44 showed the highest value of leucine in both breaker and ripe stage, but also the lowest content of sugars, whose concentration was always higher in the C3 variety. As demonstrated by Tieman et al. (2018), the level of compounds that influence the aroma in tomato fruits, vary greatly in modern and heirloom varieties and wild accessions, founding that often modern commercial varieties contain significantly lower amounts of many flavor chemicals than older varieties. The commercial variety C3 evaluated in this study, can be assimilated to the “Cherry tomatoes” category that are tastier than standard regular size tomato, justifying the higher concentration of sugar observed, especially when full ripe. This is probably due to the lower fruit size and yield per plant in Cherry tomatoes than for regular size tomato varieties (Panthee et al., 2013; Figàs et al., 2015b). Another interesting variety is the landrace P05, which showed the highest value of glutamic acid when full ripe. This compound is an amino acid that in fruits represents a flavor-enhancing compound (Oms-Oliu et al., 2011). Indeed, it is sensed as "umami" (the fifth

basic taste) evoking a savory feeling (Chaudhari et al., 2009; Baldina et al., 2016).

In this work, also the changes in carotenoids content were investigated during fruits ripening. As expected, an increase in the carotenoid content was observed, which are associated with the ripening process in tomato fruits (Bramley, 2002; Giovannoni, 2004; Giovannoni et al., 2017). The results showed a high degree of variation between the two ripening stages and the broad sense heritability (H^2) observed at the ripe stage for all carotenoids analyzed (phytoene, phytofluene, lycopene and β-carotene) suggest that the genotype is a determinant factor in affecting the carotenoid content (Baldina et al., 2016). The difference in carotenoids content between the two groups (landraces and commercial varieties) and among the accession were also investigated in both breaker and ripe stage. Due to a high range of variation of each carotenoid in the accessions, no significant differences were found between the two groups in both ripening stages. However, among the accessions, the landrace P04 stood out at the ripe stage as the richest in carotenoids contents, especially for their high lycopene content (180 mg 100 g^{-1} dried sample weigh), followed by the landraces P46 and P16 and the commercial variety C2 (*LYC* = 79.59, 76.45, 74.44 mg 100 g^{-1} dried sample weigh, respectively). This suggests that the landrace P04 could be considered as an interesting material to be used in breeding programmes aimed to develop new improved cultivars with higher nutritional values and quality (Casals et al., 2011). In the collection, the carotenoids contents with largest variation were those in lycopene and β-carotene. Apart from their interest for the functional and bioactive properties, both carotenoids are determinant for the fruit color (Abbasi et al., 2019; Siddiqui et al., 2015). The selection of local accessions with high content in lycopene would result in an added value in genetic advances and the results obtained from this collection would allow the

selection of local accessions of tomato with better quality and adapted to the demands of consumers (Panthee et al., 2013; Petropoulos et al., 2019).

The diversity for metabolite composition found in the collection of tomato local varieties evaluated, reveal that landraces are highly variable for chemical traits and, therefore, amenable to selection, as previously determined in other studies on the diversity for chemical composition of local varieties of tomato (Rodríguez-Burruezo et al., 2005; Labate et al., 2011; Panthee et al., 2013; Cortés-Olmos et al., 2014; Sacco et al., 2015). All these results, would be of great relevance for the enhancement of local varieties associated to high standards of quality or to identify sources of variation for breeding, as well as to satisfy directly both farmers and consumers needs (Rodríguez-Burruezo et al., 2005; Hurtado et al., 2014; Cortés-Olmos et al., 2014).

1.5 Conclusions

The value of this research is mainly represented by the deep characterization of the collection carried out at different levels, which aimed at the preservation, conservation and valorization of local resources, such as those of agro-biodiversity, to meet current needs (materials adapted to the cultivation and commercialization) and future needs (materials adapted to use in breeding programs) and to investigate compounds that, at the same time, could be useful in preventing human diseases. Indeed, the main objective of this work was to evaluate a representative group of tomato Sardinian landraces in comparison to commercial varieties for morpho-phenological and metabolic composition. Concurrently, a genetic diversity analysis through SNP markers was performed to describe the collection diversity.

Following the first step of study and selection that allowed to identify the most adapted varieties to the environment of cultivation and the modern horticultural techniques used, the comparison of morpho-phenological, metabolic and genetic data allowed to evaluate the potentials of some local varieties. These accessions represent a valuable material to be used for their direct valorization and the present results will also contribute to promote the *in-situ* conservation of these local tomato varieties by farmers. Indeed, the morphological, molecular, chemical and organoleptic characterization of these landraces allowed to define their distinctive characteristics, useful information to implement their use in local markets. For example, their characterization could allow the recognition of denomination, indication or quality certifications by the European Union ensuring for their unique characteristics and providing protection against imitation (Polegri and Negri, 2010; Spataro and Negri, 2013; Hurtado et al., 2014). The information obtained may also be useful to tomato breeding programs for improving multiple traits associated to the quality of tomato fruits.

An interesting strategy that can allow an active enhancement of local varieties with positive repercussions also directly on the territory, is represented by the participatory enhancement programmes. These directly involve the farmers in the characterization, selection and breeding process, contributing to the recovering of local varieties and providing added value both to landraces and farmers (Hurtado et al., 2014).

CHAPTER 2

2 Shelf-life evaluation and quality characterization of fruits

2.1 Introduction

The quality of fruits and vegetables include multiple characteristics, which depend both on physicochemical and nutraceutical properties of horticultural products, but also on the consumer perception (Kyriacou and Rouphael, 2018).

The quality of horticultural products mostly depend on genotypic, agro-environmental, harvesting and postharvest factors (Raffo et al., 2002; Javanmardi and Kubota, 2006; Majidi et al., 2011; Iglesias et al., 2015; Sánchez-González et al., 2015), but it is also influenced by socioeconomic and marketing factors which condition consumer perception of products (Causse et al., 2003; Schreiner et al., 2013). Different attributes of quality have been defined and are related to the appearance and the shelf-life of products and to the organoleptic quality (Shewfelt, 1999; Barrett et al., 2010; Shewfelt, 2014). The commercial quality mainly depends on external appearance of the products including color, shape, size, presence of defects and firmness, while the organoleptic quality depends on biochemical parameters, such as the contents in antioxidants, sugars, acids and volatile compounds, which determine the overall flavor of the fruits and their nutritional value (Bertin and Génard, 2018).

First among all, the color is one of the most important indicators of food quality and shelf-life (Batu, 2004; Radzevičius et al., 2009). The fruit color

during ripening is produced by a combination of pigments and its changes are due to loss of chlorophyll (green color) and synthesis of anthocyanins or carotenoids, such as β-carotene (orange color) (Valero and Serrano, 2013). In tomato fruits, for example, the chlorophyll imparts a green color during the early developmental stages of the fruit, then, during the ripening process, it is degraded to chromoplasts with a consequent increase of the carotenoids content. These changes lead to a very different color of the fruit, such as yellow, orange, pink and red (Arias et al., 2000; Egea et al., 2010).

The carotenoids are important not only for the color they impart to the fruit but also because they have recognized health benefits (Martí et al., 2016; Rodríguez-Concepción et al., 2018; Eggersdorfer and Wyss, 2018). In fact, numerous epidemiological studies have demonstrated that a diet rich in nutraceutical and functional food can prevent various diseases, including some cancers (*e.g.* prostate and esophagus) and cardiovascular diseases (Livingstone et al., 2017; Kulczyński et al., 2017; Abbasi et al., 2019). In the case of tomato, its demand is increasing also because of the recognized carotenoid benefits to human health which underline the importance of this vegetable in the diet (Rao and Rao, 2007, Adalid et al., 2010; Dar and Sharma, 2011).

Other nutrients and bioactive compounds, such as minerals, vitamins, essential amino acids, sugars and fiber, contribute to the nutritional value of tomato fruits (Quinet et al., 2019). These compounds are also responsible of the tomato flavor and aroma: sweetness is determined by the concentrations of the predominant sugar while sourness is determined by the concentrations of the predominant organic acids (Kader, 2008; Teka, 2013).

Other important indicators used to evaluate fruit quality are related to their physical characteristics. Texture properties, such as firmness, are the most important indicators and they can be defined like the physical and

structural food characteristics related to deformation and disintegration of food under a force (Barrett et al., 2010). The firmness corresponds to the "stiffness" and it is described by the force-deformation relationship (Lesage and Destain, 1996). In tomato, the fruit firmness depends on the cellulosic structure and cell turgor of the tissue (Huang et al., 2018) and it is related to the ripening process, susceptibility of the fruit to physical damages during harvest, maturity stage and storage potential (Lesage and Destain, 1996; Teka, 2013). Moreover, firmness is related to the weight loss, a process associated with postharvest dehydration resulting in tissue softening and turgor loss and, especially in tomato, the fruits become dull and very soft when weight loss is high (Pinheiro et al., 2013). As a consequence, the evolution of tomato fruit firmness during the maturation process affects the shelf life of the fruit and the consumer choice (Causse et al., 2010; Huang et al., 2018).

The shelf-life of products is another key characteristic associated to the postharvest quality of food. Shelf-life is the time a product can be stored without losing its qualitative characteristics and is one of the most critical quality traits for fleshy fruits (Petric et al., 2018). In particular the shelf-life of horticultural products such as tomato fruits, can be shortened due to different factors, such as the maturity stage at harvest, the postharvest conditions and the pathogens diseases which can promote over-ripening effects (Petric et al., 2018).

Tomato is a climacteric fruit that can be harvested at different ripening stages, from breaking to red ripe, but it is characterized by a high perishability (Arah et al., 2015). One of the most used approaches to extend tomato shelf life and to maintain its commercial quality and safety is to harvest the fruits at the mature green stage, followed by low-temperature storage and ethylene exposure to induce ripening. Other strategies are based on the use of natural mutants or transgenic plants having functional mutations at the level of ripening-related genes and the use of preservation technologies, such as

modified atmosphere packaging (Hong and Gross, 2001; Vrebalov et al., 2002; Moneruzzaman et al., 2009; D'Aquino et al., 2016; Zhang et al., 2017; Yu et al., 2017). However, some of these techniques, and their related different management levels, could negatively affect taste, flavor and nutritional quality and they may cause physiological disorder or chilling injury (e.g. storage temperature below 13°) (Pinheiro et al., 2013; Arah et al., 2015).

Color, texture, size and shape are the principal factors considered by consumers, but environmental components, sensory characteristics and nutritional value are becoming progressively important (Serrano-Megías and López-Nicolás, 2006; Kader, 2008). In fact, the scientific community is increasingly engaged in the research for innovative strategies to improve both quality (Sauvage et al., 2014; Tieman et al., 2017;) and commercial characteristics (Beckles, 2012; Uluisik et al., 2016; Yu et al., 2017) associating sustainable and low-input production systems in order to meet the consumer demands (Bertin and Genard,2018).

For a long time, the tomato breeding has focused on yield, fruit appearance and shelf-life leading to a large diversity in size, shape and color (Frary and Doganlar, 2000; Causse et al., 2002; Bai and Lindhout, 2007; Casals et al., 2011; Petropoulos et al., 2019). Only recently the activities of breeding programs are dealing with the improvement of both organoleptic quality and health value of tomato fruits (Petric et al., 2018). Genetic engineering approaches can be used to modify the expression of genes controlling quality attributes, but the use of genetic transformations for commercial purposes is highly regulated (Bertin and Genard, 2018). On the other side, the natural genetic variability within or among species represents a great potential for creating new improved varieties (Gur and Zamir, 2004; Causse et al., 2010; Lin et al., 2014)

The use of this biodiversity can contribute to food security and improved food nutrition (Toledo and Burlingame, 2006). The potential value of wild species and locally adapted varieties (landraces) has been recognized (Brush, 2000; McCouch et al., 2013). In particular, the landraces can have fewer disadvantages if used in genetic improvement programs compared to the wild progenitors of cultivated species (McCouch et al., 2013). Preserving this material from the risk of progressive genetic erosion and enhancing their peculiarities are therefore important objectives for the scientific community (Tuberosa et al., 2011; Davey et al., 2011).Indeed, these genetic resources possess some characteristics that can be useful to respond to the changing demands of society in terms of food production and consumption (Toledo and Burlingame, 2006; FAO, 2019).

In particular, several genetic resources of tomato are available and can be used both for research and genetic improvement. Numerous studies have been conducted with the aim of comparing the genetic variability of tomato landraces with that of modern commercial varieties. For example, Renna et al. (2018) evaluated the long-storage Italian tomato landrace “Regina”, for the main physical and chemical traits both at harvest and after three months of storage. The results indicated a high qualitative profile, especially for the concentration of tocopherols, lycopene and ascorbic acid, highlighting the interesting and unique characteristics of this tomato landrace. A total of 49 accessions of tomato from 24 countries on 4 continents were evaluated by Adalid et al. (2010) for the content of lycopene, β-carotene and ascorbic acid in order to recover their use. This work has shown the great variability in the bioactive component content of tomato fruits that can be found in underutilized cultivars, their potential use for direct human consumption, cultivation or as diversity sources in breeding programs. Garcia et al. (2016) evaluated a group of eight traditional Spain cherry tomato landraces under greenhouse conditions. Among the few landraces that emerged as valuable to

satisfy special market needs or as sources of genes to develop new varieties with higher organoleptic and nutritional quality, one stood out for yield, fruit weight, firmness, flavor, lycopene and ascorbic acid contents.

These studies represent an example of the importance to characterize local varieties which can provide useful information on a series of parameters related to the physicochemical and color characteristics of the fruit. Once evaluated their fruit's quality, this information might be used to identify potential parents adapt for breeding programs or to encourage the cultivation of interesting varieties for a particular trait (Gómez et al., 2001).

The objective of this study was to evaluate the response to storage of a group of tomato Sardinian landraces in comparison to commercial varieties (eight local varieties and two cultivars) and to investigate the changes in their quality's characteristics during 30 days of storage at refrigerated conditions. In detail, we looked for accessions characterized by a long shelf-life and/or accessions with interesting characteristics to be used in future breeding programs aimed at improving tomato quality traits.

2.2 Materials and Methods

2.2.1 Plant Materials and storage conditions

A collection of tomato (*Solanum lycopersicum* L.) Sardinian landraces and two modern varieties were grown under greenhouse conditions during the autumn-winter season for two consecutive years, 2017-2018 (EX1) and 2018-2019 (EX2). The trials were conducted on a horticultural specialized farm (Società Agricola F.lli Scintu) located in Oristano, Sardinia. All details about plant materials and trials are described in section 1.2 of Chapter 1. For both years a shelf-life trial was set up: four tomato Sardinian landraces were evaluated

in comparison to two commercial varieties during the 2017-2018 trial, while in the 2018-2019 trial six landraces were evaluated in comparison to two commercial varieties (Table 1).

Table 1: List of varieties evaluated in 2017-2018 (EX1) and 2018-2019 (EX2).

Harvesting Stage	Variety	Code	Collection[a]	EX1	EX2
Red-ripe	Arracadas	P01	L-SAR	x	x
	Lorigheddas de appiccai	P05	L-SAR	x	x
	Tamatta groga de appiccai	P44	L-SAR		x
	Datterino	C3	CV	x	x
Turning	Tamatticasa tundas a siccu	P04	L-SAR		x
	Tamatta kaki	P16	L-SAR	x	x
	Tamatta cor'e boi	P46	L-SAR	x	x
	Camone	C1	CV	x	x

[a] L-SAR= Sardinian landrace; CV = commercial variety; X = presence of the variety in the experiment.

The fruits were harvested according to the USDA standard chart for tomato color classification (USDA, 1991) and the local harvesting standards (at the different maturity stages) for marketing. Three Sardinian landraces (*Arracadas*, *Lorigheddas de appiccai* and *Tamattac groga de appiccai*) and one commercial variety (*Datterino*) were harvested at the "red-ripe stage"; three other Sardinian landraces (*Tamatta cor'eboi, Tamatta kaki* and *Tamatticasa tundas a siccu*) and one commercial variety (*Camone*) were harvested at the "turning stage". The fruits were selected for uniformity in size, color, absence of visual defects and fungal infection, and then transferred to the laboratory of University of Sassari for subsequent analyses. The fruits were stored at 13°C for 30 days and the analysis, mostly conducted according to the procedures described by D'Aquino et al. (2016), were performed at 10 days intervals from the harvest date, for a total of four inspection times (from now on indicated as T0, T10, T20, T30). The list of the parameters analyzed for each variety is shown in Table 2.

Table 2: List of analyses and parameters used to evaluate the shelf-life of the varieties both EX1 and EX2.

Type of analysis	Trait	Code	Unit of measure
Visual descriptors	Weight loss	WL	%
	Visual quality	VQ	Score (1-9)
Color parameters	Lightness	L*	
	Redness and greenness	a*	
	Yellowness and blueness	b*	
	Chroma	C	
	Hue angle	H°	
Texture parameters	Force required to puncture tomato skin	Fp	N
	Fruit deformation before skin rupture	Dp	mm
	Mechanical work necessary to reach the breaking point	Wp	N.mm
	Firmness	Ep	N/mm
Chimical analyses	pH	pH	
	Total SolubleSolids	TSS	°Brix
	TitratableAcidity	TA	gL^{-1}citric acid
	Lycopene	LYC	mg $100g^{-1}$

2.2.2 Visual quality and weight loss

Before storage, five fruits for each variety were selected to determine the weight loss (*WL*) and the overall appearance (*VQ*). The fruits were then individually weighed at harvest and at each of the four storage times. Weight loss was calculated as a % reduction of the initial weight. As indicated in D'Aquino et al. (2016), the overall appearance was determined at each storage time using a 9 points hedonic scale, where: 1 = very poor; 3 = poor; 5 = good (limit of marketability); 7 = very good; 9 = excellent. The score was based on a judgment of visual defects (shriveling, bruising, pitting, etc.) and loss of firmness perceived after a slight pressure by fingers. The end of the shelf-life was declared when the mean overall appearance for each variety was below the limit of marketability or the majority of the fruits were rotten.

2.2.3 Color assessment

For each sample, the color assessment was carried out in triplicate on five different points of the fruit equator by using a CR 300 Minolta colorimeter (Konica Minolta Sensing, Osaka, Japan). Color assessment was repeated at each storage time on the same fruits used to quantify the weight loss for each variety.

The CIE-Lab values were recorded, where the L^* represents lightness, ranging from 0 (black) to 100 (white); the a^* quantifies greenness (negative values) to redness (positive values) and b^* represents blueness (negative values) and yellowness (positive values). Before measurement, the colorimeter was calibrated with a standard white plate. Also, the chroma (C) and the hue angle ($H°$) were calculated according to the following equations (Luo, 2015):

$$(1) \qquad C = [a^{*2} + b^{*2}]^{1/2}$$

$$(2) \qquad H° = tan^{-1}[b^*/a^*]$$

The chroma, or saturation index, and the hue angle are the quantitative and qualitative attribute of color, respectively. In particular, chroma is the degree difference in comparison to a grey color with the same lightness for each hue, while the hue angle is an indicator of the chromatic nature of the color and defines the colors traditionally as reddish, yellowish and greenish (Abdelaali et al., 2018; Meléndez-Martínez et al. 2003). It is expressed in degrees and ranges from 0° to 360° (0°, 90°, 180° and 270° are pure red, yellow, green and blue colors, respectively).

2.2.4 Chemical analyzes and lycopene determination

All chemical analyzes were performed in triplicate from filtered juices obtained by the homogenization of 100 g of tomato fruits with a domestic blender. The juice was centrifuged at 9,000 rpm for 15 min, then the supernatant was filtered through a 0.45 mm cellulose acetate filter

The pH was measured using a pH-meter (Orion 420A, Thermo Fisher Scientific, Waltham, US), the total soluble solid (*TSS*) content was measured with a digital refractometer (PR-101 Palette series, ATAGO CO., LTD, Japan) and expressed as °Brix, while titratable acidity (*TA*) was calculated as gL^{-1} citric acid in the juice after titration of 5 g of juice sample with 0.1N NaOH to an endpoint of pH 8.2.

Lycopene (*LYC*) content was determined as described in Kobec et al. (2012). Briefly: 50 mL of a hexane/acetone/ethanol (2:1:1) solution was added to 5 g of the homogenized fresh tomato sample. The solution was then kept in agitation into a vial glass wrapped with an aluminum foil to exclude light. After 60 min the non-polar layer containing lycopene was measured at 470 nm by an UV–vis spectrophotometer (Spectrophotometer mod. 8453, Hewlett–Packard, Palo Alto, California). A calibration curve, obtained using different standard lycopene concentrations, was set up and then used to determine the content of total lycopene (mg 100 g^{-1} fresh fruit).

2.2.5 Texture assessments

Texture analyses were conducted in fruits using a testing machine (TA. XT2 Plus Texture Analyzer, Stable Micro Systems, Surrey, UK) and texture

parameters were automatically computed by the Texture Exponent 32 software (Stable Microsystems, Surrey, U.K.). A penetration test was performed using three fruits for each variety and storage time, to monitor changes during the storage of the samples. For the puncture test, the fruits were placed on a platform and punctured in the lateral face by a needle probe of 2 mm in diameter. A final depth of 1.5 cm at a speed of 1.7 mm s^{-1} was reached and the applied force (expressed in Newtons) was recorded. Four parameters were registered: the force required to puncture tomato skin (*Fp*, [N]) that represents the skin rupture; the probe position at *Fp* (*Dp*, [mm]) which determines fruit deformation before the skin rupture; the mechanical work necessary to reach the breaking point (*Wp*, [N.mm]) that is the area under the curve up to the skin rupture point; the firmness (*Ep*, [N mm^{-1}]) which represents the slope of the first part of the curve, defined as *Fp/Dp* (Figure 1; Camps, 2018).

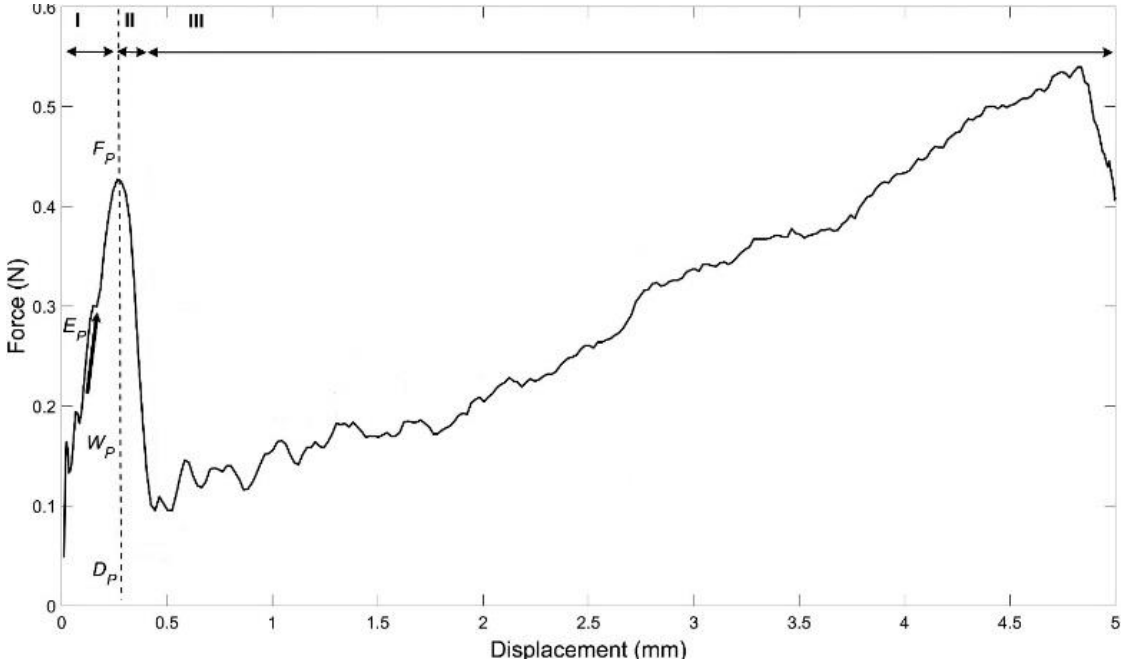


Figure 1: Representative graph and parameters of a penetrometry test. Source: Camps (2018) (Modified).

2.2.6 Statistical analysis

All the statistical analyses were performed by using JMP 7 (SAS Institute, Inc.). Collected data were analyzed by one-way analysis of variance (ANOVA). To verify if significant differences were present among varieties within storage time, the varieties were selected as the factor; to evaluate changes during the conservation, the storage time was chosen as the factor.

Means were separated according to the Tukey honest significant difference (HSD) at $p<0.05$ level. Graphics were created using MS-Excel 2016.

A multivariate analysis was used to obtain an overview of the whole data variability. The Principal Component Analysis (PCA) was performed on mean data matrix for the two groups (Turning and Red-ripe). Fifteen parameters and 12 observations (three varieties and four storage times) were used for EX1 and 16 observations (four varieties and four storage times) for EX2. The principal components (PCs) with eigenvalues ≥1 were retained for data discussion (Dunteman, 1989) and the correlations between each parameter and the PCs were calculated; loadings greater than |0.6| were then considered for further discussion. The results of the PCA are shown as biplots of scores (varieties x storage time) and loadings (variables), drawn by using RStudio 1.1.423 (RStudio, Inc. 2016, Boston, MA, USA).

2.3 Results

2.3.1 Visual quality and weight loss

The overall shelf-life of the two groups of Sardinian tomato landraces as evaluated in the two years by the visual quality of the fruits has evidenced different results (Fig. 2). The end of the shelf-life was declared when the mean overall appearance for each variety was below the limit of marketability (5 point) and when most of the fruits were rotten (Tab. 3).

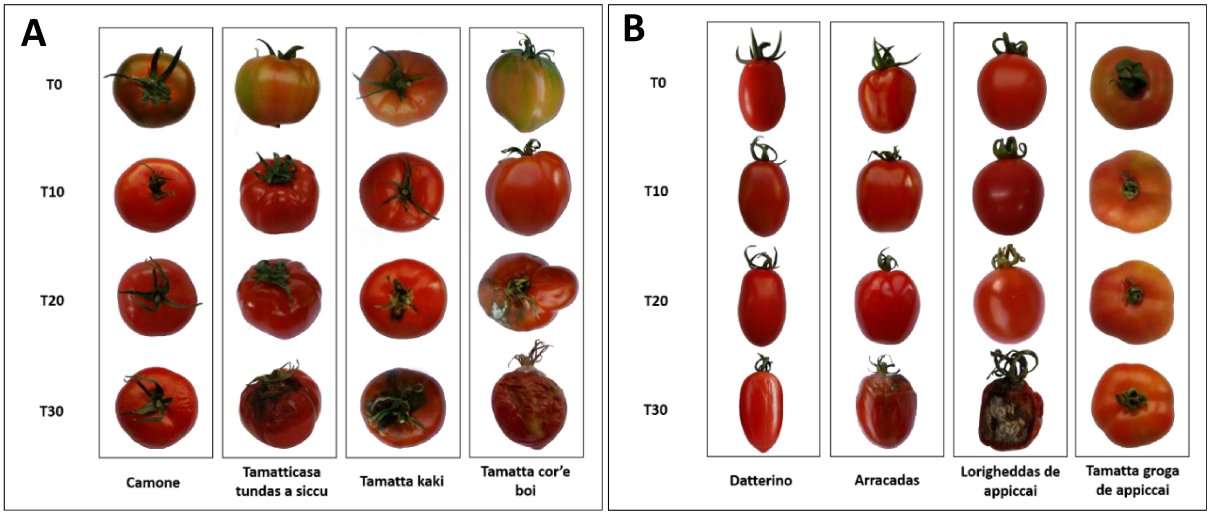


Figure 2: Changes in shelf-life of tomato fruits within the turning group (A) and the red-ripe group (B) as registered during the EX1 experiment.

Note: T10 = tend days of storage; T20 = twenty days of storage; T30: thirty days of storage.

Table 3: Shelf-life of varieties within the turning and red-ripe group in both experiments.

Group	Variety	EX1			EX2		
		T10	T20	T30	T10	T20	T30
Turning	Tamatticasa tundas a siccu	-	-	-	x		
	Tamatta kaki	x	x		x		
	Tamatta cor'e boi	x	x		x		
	Camone	x			x		
Red-ripe	Arracadas	x			x	x	
	Lorigheddas de appiccai	x			x		
	Tamatta groga de appiccai	-	-	-	x	x	
	Datterino	x	x		x	x	x

Note: T10 = tend days of storage; T20 = twenty days of storage; T30: thirty days of storage.
X = presence of the variety in the storage time.

During EX1 none of the varieties reached 30 days of storage in both groups (Tab. 3). Within the turning group, only the two landraces reached 20 days of storage, while the shelf-life of the commercial variety Camone ended up at T10 due to the appearance of various pathogens on the fruit skin (Tab. 3). In detail, the visual quality (*VQ*) was unchanged at T10 for the landrace Tamatta kaki (*VQ* = 9) and the commercial variety Camone (*VQ* = 9), while it slightly declined (*VQ* = 7) for the landrace Tamatta cor'e boi (Fig. 3A). After 20 days, the score of Tamatta kaki declined to *VQ* = 6.2 showing only negligible differences from Tamatta cor'e boi (Fig. 3A).

On the contrary, in the red-ripe group only the commercial variety Datterino reached a shelf-life of 20 days (*VQ* = 6,4) while the two Sardinian landraces already at T10 showed a high percentage of fruits at the limit of marketability (Arracadas *VQ* = 5 and Lorigheddas de appiccai *VQ* = 5.8) (Fig. 3C).

During EX2 the shelf-life for all turning group's varieties ended up after 10 days of storage (Tab. 3): the overall appearance for all the accessions declined very rapidly, especially for Tamatticasa tundas a siccu and Tamatta cor'e boi that showed a high percentage of fruits at the limit of marketability (*VQ* = 5) (Fig. 3B).

Within the red-ripe group, the commercial variety Datterino showed the most extended shelf-life (30 days), followed by the landraces Arracadas and Tamatta groga de appiccai (20 days) and the landrace Lorigheddas de appiccai (10 days) (Tab. 3). In detail: at T10 the commercial variety Datterino and the landrace Tamatta groga de appiccai showed an unchanged visual quality from their initial freshness, while the quality score declined for the other two landraces (Arracadas *VQ* = 7; Lorigheddas de appiccai *VQ* = 5). At T20 the appearance of the fruit worsened, especially for the landraces Arracadas (*VQ* = 5) and Tamatta groga de appiccai (*VQ* = 5), which were significantly different from the control (Datterino). After 30 days of storage the fruits of the

commercial variety Datterino were still completely marketable (*VQ* = 8.2) (Fig. 3D).

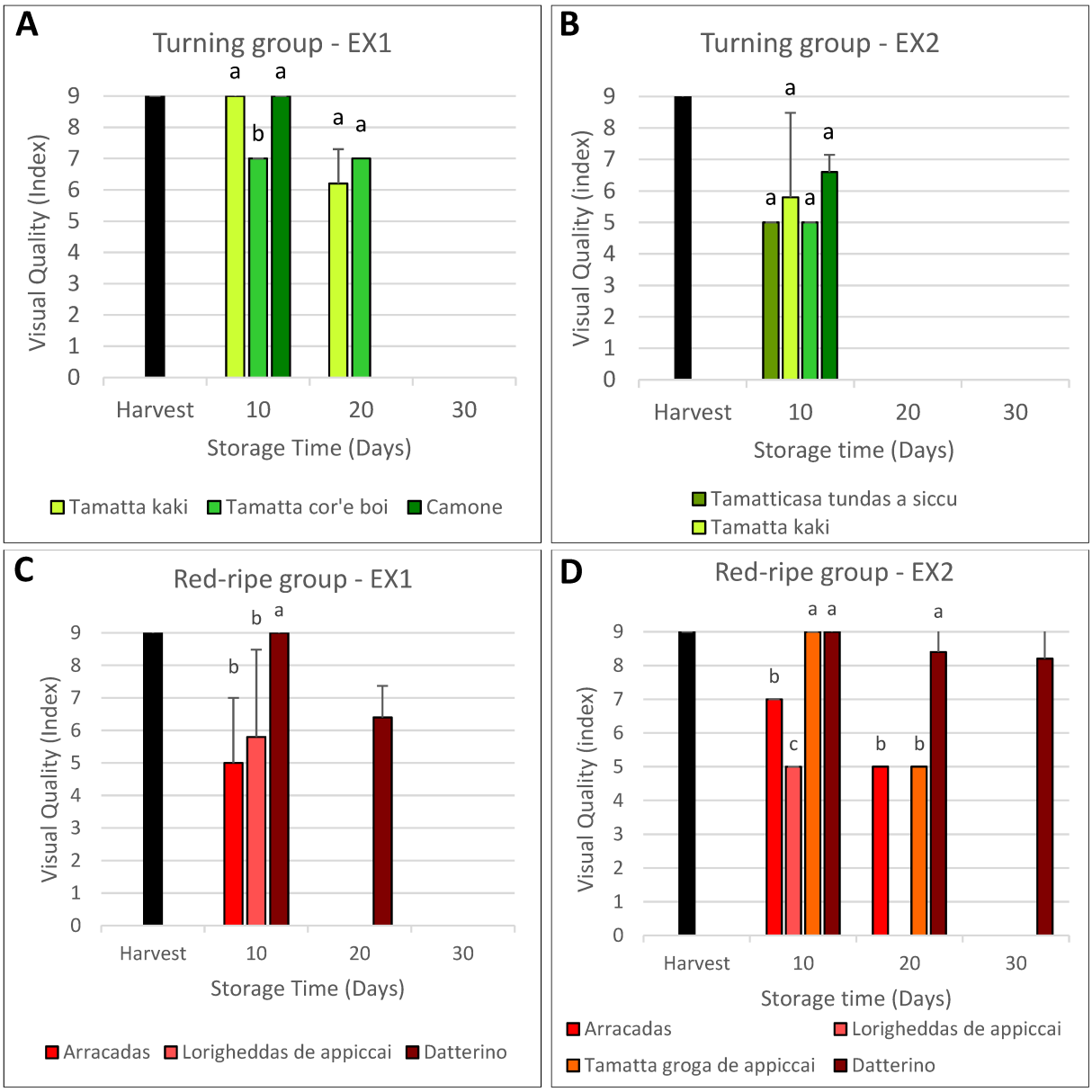


Figure 3: Visual quality of the varieties in turning group in EX1 (A) and EX2 (B) and in red-ripe group in EX1 (C) and EX2 (D).

Harvest black bar indicate the visual quality (Index = 9) of all varieties at the harvest time. Means followed by different letters indicate significant differences among the varieties within each storage time based on Tukey-Kramer's test at $p < 0.05$. Vertical bars represent the standard deviation.

Based on this preliminary quality assessment, in the following sections it will illustrate the results for each variety as obtained from harvest until the respective shelf-life's end.

Weight loss (*WL*) changes in turning group's fruits appeared overall lower than those measured in the red-ripe group's fruits (Fig. 4).

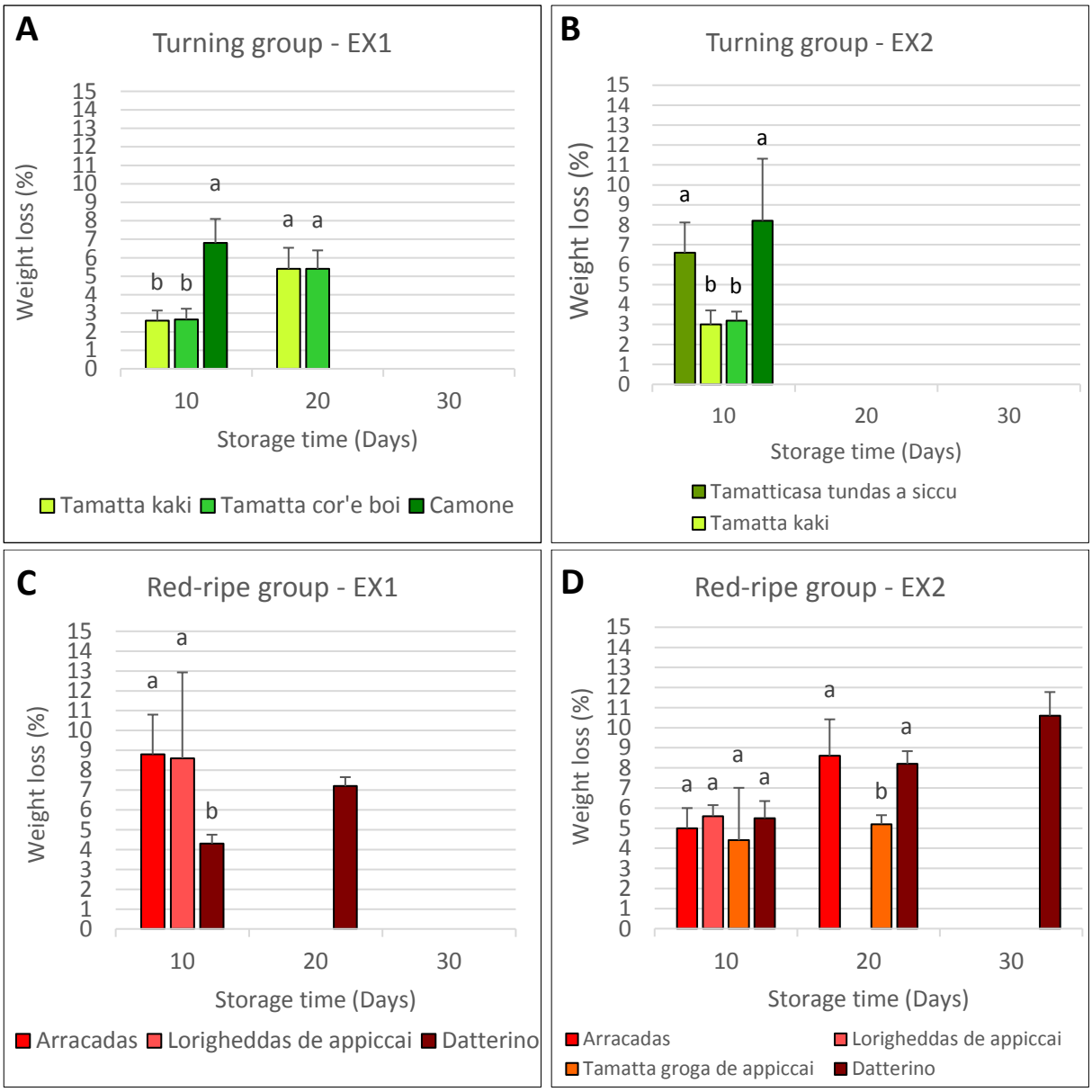


Figure 4: Weight loss (% reduction of the initial weight) in the varieties of both turning group in the EX1 (A) and EX2 (B) and red-ripe group in the EX1 (C) and EX2 (D).

Means followed by different letters indicate significant differences among the varieties within each storage time based on Tukey-Kramer's test at $p < 0.05$. Vertical bars represent the standard deviation.

Regarding the turning group in EX1, at T10 the weight loss was significantly different between the landraces and the commercial variety

which showed the highest weight loss (6,8%); instead at T20 there was no difference between the two landraces both showing a weight loss of 5,4% (Fig. 4A). In EX2, the commercial variety and the landrace Tamatticasa tundas a siccu showed the higher WL at T10 (8,2% and 6,6%, respectively) with no significant differences between them. The other two accessions, Tamatta kaki and Tamatta cor'e boi, similarly lost about the 3% of their weight (Fig. 4B).

For the accessions harvested at the red-ripe stage, in EX1 the two Sardinian landraces Arracadas and Lorigheddas de appiccai showed the highest WL at T10 (8,8% and 8,6%, respectively; Fig. 4C). At T10 there was no significant difference among all the red-ripe group's accessions while at T20 the landrace Tamatta groga de appiccai showed a significant lower weight loss in respect to Arraccadas and Datterino and it retained almost the same WL as at T10 (Fig. 4D).

2.3.2 Color determination

Color parameters for all of the experimental trials are shown in Tables 4 and 5. In the turning group varieties, we observed for both years a decrease of *L** (lightness) and *b** (blueness-yellowness) values and an increase of the *a** (greenness-redness) values during the storage. These changes resulted in an increase of redness and a decrease of yellowness in the fruits color due to the ripening process (Tab. 4). Chroma values slightly increased during the storage, with no significant differences among sampling times. The only exceptions were the landrace Tamatta kaki and the commercial variety Camone that during the EX2, showed an increase of the strength of the color at T10 (Tab. 4). On the contrary, the Hue angle (*H°)* decreased during the storage in both years, with significant differences among sampling times for almost all of the varieties (Tab. 4). An exception was the landrace Tamatta kaki that in EX1

showed an increase of the reddish color of the fruit (*H°* = 0° indicates A completely red color).

Table 4: Color parameters as registered within the turning group in both experiments.

Turning Group					
EX1					
Varieties	**L***	**a***	**b***	**C**	**H°**
T0					
Tamatta kaki	49.66 ± 1.29 a A	20.03 ± 1.22 a B	32.10 ± 1.04 a A	37.86 ± 1.15 a B	58.04 ± 1.69 b A
Tamatta cor'e boi	52.12 ± 1.13 a A	19.08 ± 2.65 a B	31.86 ± 0.35 a A	37.20 ± 1.05 a A	59.16 ± 3.80 b A
Camone	42.27 ± 1.28 a A	8.31 ± 0.89 b B	23.97 ± 0.71 b A	25.38 ± 0.78 b B	70.90 ± 1.88 a A
T10					
Tamatta kaki	44.26 ± 1.89 a B	26.72 ± 1.68 a A	29.77 ± 1.67 a AB	40.05 ± 0.41 a A	48.08 ± 3.34 ab A
Tamatta cor'e boi	44.81 ± 0.58 a B	28.39 ± 1.97 a A	28.75 ± 0.32 a B	40.42 ± 1.56 a A	45.40 ± 1.78 b B
Camone	39.93 ± 0.71 b B	18.28 ± 1.53 b A	22.98 ± 0.89 b A	29.38 ± 1.29 b A	51.54 ± 2.34 a B
T20					
Tamatta kaki	42.06 ± 1.34 a B	27.90 ± 1.07 a A	27.36 ± 1.64 a B	39.10 ± 1.50 a AB	44.41 ± 1.83 a B
Tamatta cor'e boi	42.24 ± 0.97 a C	28.91 ± 1.65 a A	26.92 ± 1.96 a B	39.50 ± 2.53 a A	42.94 ± 0.62 a B
Camone	-	-	-	-	-
T30					
Tamatta kaki	-	-	-	-	-
Tamatta cor'e boi	-	-	-	-	-
Camone	-	-	-	-	-
EX2					
Varieties	**L***	**a***	**b***	**C**	**H°**
T0					
Tamatticasa tundas a siccu	51.44 ± 1.38 a A	5.37 ± 1.25 b B	27.19 ± 2.26 a A	27.74 ± 2.12 a B	78.72 ± 3.06 a A
Tamatta kaki	53.21 ± 1.65 a A	12.61 ± 0.90 a B	41.04 ± 1.73 a A	43.26 ± 23.87 a A	70.12 ± 6.46 ab A
Tamatta cor'e boi	51.40 ± 0.64 a A	11.99 ± 3.39 a B	26.83 ± 3.24 a A	29.49 ± 3.82 a A	66.16 ± 5.40 b A
Camone	41.83 ± 2.11 b A	6.39 ± 2.00 b B	24.87 ± 2.21 a A	25.74 ± 2.29 a A	75.68 ± 4.42 a A
T10					
Tamatticasa tundas a siccu	42.95 ± 1.01 a B	21.23 ± 0.46 a A	25.47 ± 1.33 ab A	33.16 ± 3.55 ab A	50.26 ± 1.72 a B
Tamatta kaki	43.79 ± 0.99 a B	23.69 ± 2.66 a A	27.64 ± 2.54 a A	36.41 ± 1.80 a A	49.37 ± 1.36 a B
Tamatta cor'e boi	42.58 ± 1.26 a B	21.71 ± 1.00 a A	21.71 ± 1.73 bc B	30.71 ± 1.24 b A	44.95 ± 1.26 a B
Camone	38.81 ± 1.70 b B	20.48 ± 3.32 a A	21.27 ± 2.81 c A	29.65 ± 3.08 b A	46.20 ± 5.88 a B
T20					
Tamatticasa tundas a siccu	-	-	-	-	-
Tamatta kaki	-	-	-	-	-
Tamatta cor'e boi	-	-	-	-	-
Camone	-	-	-	-	-
T30					
Tamatticasa tundas a siccu	-	-	-	-	-
Tamatta kaki	-	-	-	-	-
Tamatta cor'e boi	-	-	-	-	-
Camone	-	-	-	-	-

Data correspond to the mean ± SD of five independent replicates. Means followed by different letters indicate significant differences among the varieties within each storage time (lowercase letters) and among storage times within variety (capital letters) based on Tukey-Kramer's test at $p < 0.05$.

Note: L* = lightness; a* = redness-greenness; b* = yellowness-blueness; C = chroma; H° = hue angle; T0 = harvest; T10 = ten days; T20 = twenty days; T30 = thirty days

We also evaluated the difference among genotypes between years. In EX1 we detected significant differences among accessions for all of the color parameters at each storage time, except for *L** at harvest (Tab. 4). In particular, the commercial variety Camone had the lowest redness and yellowness at harvest (*a** = 8.31 and *b** = 23.97) and at 10 days of storage (*a** = 18.28 and *b** = 22.98) as well as it showed the lowest color intensity (*C* = 25.38 at harvest and *C* = 29.38 at T10; Tab. 4).

In EX2 significant differences were found among accessions at harvest (for *L**, *a** and *H*° values) and at T10 (for the *L**, *b**, *C* values). As previously observed in EX1, the commercial variety Camone showed the lowest lightness values at both harvest and T10, while no significant differences were found among landraces. The landraces Tamatta kaki and Tamatta cor'eboi recorded the highest values of redness at harvest (*a** = 12.61 and 11.99, respectively) resulting significantly different from the landrace Tamatticasa tundas a siccu (*a** = 5.37) and the commercial variety Camone (*a** = 6.39). In particular, the landrace Tamatta cor'e boi was the reddish one at harvest also according to the H° value (66.16°). At T10 the landrace Tamatta kaki recorded the highest C (36.41) followed by Tamatticasa tundas a siccu (33.16), Tamatta cor'e boi (30.71) and Camone (29.65) (Tab. 4).

Regarding the red-ripe group, we did not detect changes in the color characteristics during the storage in EX1, with the exception of the commercial variety Datterino which showed significantly lower *b** values across storage times (Tab. 5). In EX2, we observed a decrease of the *L** values and an increase of the *a** values across storage times for both the landrace Arracadas and Tamatta groga de appiccai; the commercial variety Datterino only showed an increase in redness at T30, due to the over ripening of the fruits (Tab. 5). Chroma values increased during the storage for all of the varieties. We observed significant differences among storage times for the landraces Arracadas and Tamatta groga de appiccai, which showed a decrease of the *H°*

parameter and a consequent increase of the redness of the fruits at T10 (Tab. 5).

Table 5: Color parameters as registered within the red-ripe group in both experiments.

Red-ripe Group					
EX1					
Varieties	**L***	**a***	**b***	**C**	**H°**
T0					
Arracadas	39.99 ± 1.62 a A	25.00 ± 1.55 ab A	21.47 ± 2.17 a aA	32.97 ± 2.44 ab A	40.59 ± 1.84 a A
Lorigheddas de appiccai	38.84 ± 2.16 ab A	23.64 ± 3.28 b A	21.91 ± 2.87 a A	32.24 ± 4.29 b A	42.85 ± 1.33 a A
Datterino	37.09 ± 0.76 b A	26.80 ± 1.05 a A	23.70 ± 1.29 a A	35.80 ± 1.06 a A	41.47 ± 2.05 a A
T10					
Arracadas	40.58 ± 5.05 a A	25.12 ± 2.11 a A	20.07 ± 1.95 b A	32.17 ± 2.80 b A	38.61 ± 1.19 b A
Lorigheddas de appiccai	37.73 ± 1.31 a A	22.27 ± 2.60 b A	19.71 ± 2.00 b A	29.74 ± 3.25 b A	41.56 ± 1.00 a A
Datterino	37.70 ± 1.05 a A	27.17 ± 0.90 a A	23.08 ± 1.37 a AB	35.67 ± 1.01 a A	40.33 ± 2.08 ab A
T20					
Arracadas	-	-	-	-	-
Lorigheddas de appiccai	-	-	-	-	-
Datterino	37.48 ± 0.97 A	26.72 ± 1.03 A	22.26 ± 1.17 B	34.79 ± 1.27 A	39.78 ± 1.47 A
T30					
Arracadas	-	-	-	-	-
Lorigheddas de appiccai	-	-	-	-	-
Datterino	-	-	-	-	-
EX2					
Varieties	**L***	**a***	**b***	**C**	**H°**
T0					
Arracadas	41.40 ± 1.28 b A	20.33 ± 1.03 b B	22.86 ± 1.14 b A	30.62 ± 0.54 b A	48.34 ± 2.70 b A
Lorigheddas de appiccai	38.89 ± 1.34 b A	17.08 ± 1.12 b A	18.25 ± 1.22 c A	25.00 ± 1.62 c A	46.89 ± 0.69 b A
Tamatta groga de appiccai	50.76 ± 2.75 a A	9.83 ± 3.13 c B	32.09 ± 3.79 a A	33.75 ± 2.88 b A	72.55 ± 6.62 a A
Datterino	39.19 ± 1.18 b AB	29.31 ± 1.90 a A	24.29 ± 2.01 b A	38.09 ± 2.41 a A	39.64 ± 2.02 c A
T10					
Arracadas	37.40 ± 0.66 b B	22.54 ± 0.76 b A	19.99 ± 0.77 b B	30.13 ± 1.00 b A	41.56 ± 0.77 bc B
Lorigheddas de appiccai	36.92 ± 1.06 b B	16.64 ± 1.47 c A	17.08 ± 1.14 c A	23.87 ± 1.31 c A	45.78 ± 3.14 b A
Tamatta groga de appiccai	45.16 ± 1.63 a B	17.65 ± 3.20 c A	23.73 ± 1.48 a B	29.72 ± 1.23 b B	53.51 ± 6.34 a B
Datterino	38.32 ± 1.34 b B	28.36 ± 1.71 a A	24.12 ± 1.94 a A	37.25 ± 2.24 a A	50.35 ± 1.99 c A
T20					
Arracadas	37.66 ± 0.21 b B	16.74 ± 0.49 b C	14.98 ± 0.46 b C	22.46 ± 0.57 c B	41.81 ± 0.93 b B
Lorigheddas de appiccai	-	-	-	-	-
Tamatta groga de appiccai	45.99 ± 1.15 a B	16.71 ± 2.23 b A	21.59 ± 0.92 a B	27.34 ± 1.51 b B	52.38 ± 3.88 a B
Datterino	37.78 ± 1.51 b B	27.08 ± 2.17 a A	22.23 ± 1.98 a A	35.04 ± 2.92 a A	39.37 ± 0.45 b A
T30					
Arracadas	-	-	-	-	-
Lorigheddas de appiccai	-	-	-	-	-
Tamatta groga de appiccai	-	-	-	-	-
Datterino	40.36 ± 1.35 A	23.27 ± 2.27 B	18.95 ± 2.07 B	30.00 ± 3.06 B	39.13 ± 0.46 A

Data correspond to the mean ± SD of five independent replicates. Means followed by different letters indicate significant differences among the varieties within each storage time (lowercase letters) and among storage times within variety (capital letters) based on Tukey-Kramer's test at $p < 0.05$.

Note: L* = lightness; a* = redness-greenness; b* = yellowness-blueness; C = chroma; H° = hue angle
T0 = harvest; T10 = ten days; T20 = twenty days; T30 = thirty days

We also found interesting differences among accessions during EX2. In particular, the landrace Tamatta groga de appiccai showed the highest lightness (*L**) values across storage times, being significantly different from the other varieties. This local variety also showed the highest yellowness (positive *b** values) from harvest (*b** = 32.09) to T20 (*b** = 21.59), with no significant differences from the commercial variety Datterino. It also had the lowest redness (positive *a** values) with no significant differences from the landrace Lorigheddas de appiccai at T10 and the landrace Arracadas at T20 (Tab. 5). On the other hand, the commercial variety Datterino showed the highest *C* and the lowest H° values at each storage time, being the most reddish variety with the highest color intensity, followed by the landrace Arracadas and the landrace Lorigheddas de appiccai (Tab. 5).

2.3.3 Chemical analyzes

Data related to pH, titratable acidity (*TA*), total soluble solid (*TSS*) content and lycopene (*LYC*) content are shown in Table 6 (turning group in both years) and Table 7 (red-ripe group in both years).

Within the turning group, the *pH* values of the tomato fruits increased with small and inconsistent differences among storage times in EX1, with the exception of the landrace Tamatta cor'eboi, which *pH* significantly increased from 4.31 at harvest to 4.45 at T20. Significant differences were also observed among varieties within storage time. Indeed, the commercial variety Camone showed the lowest pH values until T10 (4.21) while at T20 data were only available for the two landraces Tamatta kaki and Tamatta cor'e boi which showed significantly different acidity (Tab. 6). No significant changes were registered for *pH* during the storage of the different varieties in EX2 (Tab. 6), while significant divergences were registered among varieties within storage times being the commercial variety Camone the most acid at harvest (pH =

4.22) and the landrace Tamatticasa tundas a siccu the most acid at T10 (pH = 4.22).

Table 6: Chemical parameters and lycopene content as registered within turning group in both experiments.

Turning Group				
EX1				
Varieties	**pH**	**TSS**	**TA**	**LYC**
T0				
Tamatta kaki	4.23 ± 0.04 ab A	4.2 ± 0.45 a A	4.5 ± 1.15 a A	4.28 ± 1.19 ab C
Tamatta cor'e boi	4.31 ± 0.08 a B	4.1 ± 0.25 a A	4.0 ± 0.31 a A	5.48 ± 0.60 a B
Camone	4.13 ± 0.06 b A	4.0 ± 0.10 a A	4.4 ± 0.21 a A	2.62 ± 0.12 b A
T10				
Tamatta kaki	4.32 ± 0.02 a A	3.7 ± 0.57 a A	4.4 ± 0.40 ab A	6.69 ± 0.55 b B
Tamatta cor'e boi	4.31 ± 0.02 ab B	4.1 ± 0.51 a A	3.7 ± 0.23 b A	9.06 ± 0.55 a A
Camone	4.21 ± 0.02 b A	4.2 ± 0.17 a A	4.8 ± 0.15 a A	3.40 ± 0.68 c A
T20				
Tamatta kaki	4.34 ± 0.03 b A	3.4 ± 0.12 a A	4.1 ± 0.38 a A	9.05 ± 0.71 a A
Tamatta cor'e boi	4.45 ± 0.01 a A	3.6 ± 0.26 a A	3.1 ± 0.06 b B	8.59 ± 0.64 a A
Camone	-	-	-	-
T30				
Tamatta kaki	-	-	-	-
Tamatta cor'e boi	-	-	-	-
Camone	-	-	-	-
EX2				
Varieties	**pH**	**TSS**	**TA**	**LYC**
T0				
Tamatticasa tundas a siccu	4.32 ± 0.03 a A	3.8 ± 0.00 bA A	2.8 ± 0.51 c A	2.99 ± 0.28 a B
Tamatta kaki	4.28 ± 0.02 ab A	3.9 ± 0.17 b A	4.0 ± 0.40 b A	2.18 ± 0.01 b B
Tamatta cor'e boi	4.32 ± 0.05 a A	3.9 ± 0.17 b A	3.3 ± 0.36 bc A	2.94 ± 0.32 a B
Camone	4.22 ± 0.01 b A	4.7 ± 0.10 a A	5.2 ± 0.23 a A	3.38 ± 0.32 a B
T10				
Tamatticasa tundas a siccu	4.22 ± 0.05 b A	3.7 ± 0.38 b A	3.4 ± 0.66 ab A	5.34 ± 0.53 a A
Tamatta kaki	4.27 ± 0.01 ab A	3.6 ± 0.20 b A	4.4 ± 0.36 a A	6.33 ± 1.97 a A
Tamatta cor'e boi	4.33 ± 0.03 a A	3.4 ± 0.50 b A	2.8 ± 0.44 b A	5.88 ± 0.50 a A
Camone	4.25 ± 0.05 ab A	4.8 ± 0.15 a A	4.0 ± 0.23 a B	7.07 ± 0.40 a A
T20				
Tamatticasa tundas a siccu	-	-	-	-
Tamatta kaki	-	-	-	-
Tamatta cor'e boi	-	-	-	-
Camone	-	-	-	-
T30				
Tamatticasa tundas a siccu	-	-	-	-
Tamatta kaki	-	-	-	-
Tamatta cor'e boi	-	-	-	-
Camone	-	-	-	-

Data correspond to the mean ± SD of five independent replicates. Means followed by different letters indicate significant differences among the varieties within each storage time (lowercase letters) and among storage times within variety (capital letters) based on Tukey-Kramer's test at $p < 0.05$.

Note: TSS = Total Soluble Solids (°Brix); TA = Titratable Acidity (as citric acid, g L-1 juice); LYC = Lycopene (mg 100 g^{-1} fresh fruit); T0 = harvest; T10 = ten days; T20 = twenty days; T30 = thirty days.

In both years, *TSS* did not show considerable variations neither across storage times nor among genotypes at each storage time, except for the commercial variety Camone in EX2, which had the highest *TSS* values at harvest (4.7 °Brix) and at T10 (4.8 °Brix; Tab. 6).

The *TA* in EX1 ranged from 4.0 to 4.5 at harvest with no significant differences among genotypes, this slightly decreased during the storage times with no appreciable variations, except for the commercial variety Camone (Tab. 6). The same trend was observed in EX2, but significant differences were detected among genotype at harvest and at T10: at harvest, the commercial variety Camone and the landrace Tamatticasa tundas a siccu showed the highest (*TA* = 5.2) and the lowest (*TA* = 2.8) values, respectively, while at T10 the landrace Tamatta kaki and the commercial variety Camone recorded the highest values (*TA* = 4.4 and 4.0, respectively) and the landrace Tamatta cor'e boi showed the lowest value (*TA* = 2.8) (Tab. 6).

The LYC changed during storage, with significant differences among storage times and genotypes in both years (Tab. 6). In detail, results from EX1 evidenced that the landrace Tamatta cor'e boi had the highest values (*LYC* = 5.48) at harvest while the commercial variety Camone had the lowest content both at harvest (*LYC* = 2.62) and at T10 (LYC = 3.40; Tab. 6). On the contrary, in EX2 the landrace Tamatta kaki showed the lowest value (*LYC*= 2.18) at harvest, followed by the other two landraces and the commercial variety Camone (*LYC* = 2.94, 2.99, 3.38, respectively). The lycopene content increased during the conservation, with no significant differences among genotype after 10 days of storage (Tab. 6).

In the red-ripe group, null or modest significant variations were seen among accessions in EX1 for all the parameters (*pH, TA, TSS, LYC*). The only exceptions were observed for TSS for which the highest values were always observed for the commercial variety Datterino (mean 6.5 °Brix; Tab. 7) and the *LYC*, both across storage times and among varieties within storage time.

Indeed, at harvest the commercial variety Datterino showed the highest value (*LYC*= 10.60) followed by the landraces Lorigheddas de appiccai (*LYC*= 8.11) and Arracadas (*LYC*= 6.39); at T10 the same trend was observed, with the highest value recorded for the commercial variety Datterino (*LYC*= 13.13) and the lowest for the landrace Arracadas (*LYC*= 5.33; Tab. 7).

Also, in EX2 the *pH* values were quite stable among varieties with the exception of the commercial variety Datterino in which increasing acidity levels were observed from harvest (*pH*= 4.34) to T30 (*pH*= 4.55; Tab. 7). The commercial variety Datterino also showed the highest *pH* values at each storage time (Tab. 7).

The *TSS* slightly changed during the storage with no significant differences, except for the landrace Tamatta groga de appiccai, which had the highest value at harvest (4.9 °Brix) and the lowest values at T10 and T20 (4.2 and 4.1 °Brix, respectively; Tab. 7). The commercial variety Datterino showed the highest *TSS* values at each storage time (mean *TSS*=7.6 °Brix), followed by the landraces. Among the landraces, the highest values of *TSS* were recorded for Tamatta groga de appiccai and Lorigheddas de appiccai both at harvest (4.9 and 4.8 °Brix, respectively) and at T10 (4.2 and 4.6 °Brix, respectively; Tab. 7).

The highest *TA* was registered at the harvest for the commercial variety Datterino (*TA*= 5.7) which was significantly distinct from the landraces. We also detect significant variations overtime for the commercial variety Datterino, whose values decreased from 5.7 at harvest to 4.3 at T30. The *LYC* in the red-ripe group in EX2 increased during the storage with no significant differences among times, except for the landrace Arracadas. The commercial variety Datterino showed the highest LYC values at each storage time, always followed by the landraces' group.

Table 7: Chemical parameters and lycopene content as registered within red-ripe group in both experiments.

Red-ripe Group																
EX1																
Varieties	**pH**				**TSS**				**TA**				**LYC**			
T0																
Arracadas	4.25 ±	0.04	a	A	4.0 ±	0.10	b	A	5.0 ±	0.55	a	A	6.39 ±	1.51	b	A
Lorigheddas de appiccai	4.24 ±	0.04	a	A	4.5 ±	0.06	b	A	4.8 ±	0.52	a	A	8.11 ±	1.44	ab	A
Datterino	4.29 ±	0.02	a	A	6.5 ±	0.32	a	A	4.5 ±	0.29	a	A	10.60 ±	0.75	a	B
T10																
Arracadas	4.27 ±	0.02	a	A	4.1 ±	0.20	b	A	4.9 ±	0.56	a	A	5.33 ±	0.47	c	A
Lorigheddas de appiccai	4.26 ±	0.02	a	A	4.7 ±	0.92	b	A	4.9 ±	0.56	a	A	8.26 ±	1.67	b	A
Datterino	4.31 ±	0.02	a	A	6.6 ±	0.25	a	A	4.5 ±	0.12	a	A	13.13 ±	0.70	a	AB
T20																
Arracadas	-				-				-				-			
Lorigheddas de appiccai	-				-				-				-			
Datterino	4.34 ±	0.03		A	6.5 ±	0.17		A	4.5 ±	0.12		A	13.43 ±	1.43		A
T30																
Arracadas	-				-				-				-			
Lorigheddas de appiccai	-				-				-				-			
Datterino	-				-				-				-			
EX2																
Varieties	**pH**				**TSS**				**TA**				**LYC**			
T0																
Arracadas	4.25 ±	0.01	b	A	4.0 ±	0.06	c	A	4.1 ±	0.15	b	A	4.48 ±	0.53	b	B
Lorigheddas de appiccai	4.30 ±	0.04	ab	A	4.8 ±	0.25	b	A	4.3 ±	0.25	b	A	5.68 ±	1.17	b	A
Tamatta groga de appiccai	4.29 ±	0.02	ab	A	4.9 ±	0.12	b	A	4.2 ±	0.31	b	A	4.67 ±	1.49	b	A
Datterino	4.34 ±	0.02	a	C	7.6 ±	0.23	a	A	5.7 ±	0.20	a	A	14.58 ±	0.95	a	A
T10																
Arracadas	4.22 ±	0.02	b	A	3.5 ±	0.26	c	A	3.8 ±	0.31	a	A	5.45 ±	0.50	b	B
Lorigheddas de appiccai	4.25 ±	0.04	b	A	4.6 ±	0.36	b	A	4.0 ±	0.68	a	A	6.50 ±	2.20	b	A
Tamatta groga de appiccai	4.25 ±	0.02	b	A	4.2 ±	0.35	bc	B	5.3 ±	2.14	a	A	5.80 ±	1.10	b	A
Datterino	4.38 ±	0.03	a	C	7.7 ±	0.12	a	A	5.3 ±	0.15	a	AB	11.22 ±	2.59	a	A
T20																
Arracadas	4.19 ±	0.04	b	A	3.7 ±	0.23	b	A	4.1 ±	1.12	a	A	8.17 ±	0.31	b	A
Lorigheddas de appiccai	-				-				-				-			
Tamatta groga de appiccai	4.25 ±	0.04	b	A	4.1 ±	0.15	b	B	4.1 ±	0.20	a	A	6.48 ±	0.82	b	A
Datterino	4.48 ±	0.02	a	B	7.7 ±	0.20	a	A	4.9 ±	0.32	a	BC	14.70 ±	1.68	a	A
T30																
Arracadas	-				-				-				-			
Lorigheddas de appiccai	-				-				-				-			
Tamatta groga de appiccai	-				-				-				-			
Datterino	4.55 ±	0.03		A	7.5 ±	0.21		A	4.3 ±	0.26		C	10.72 ±	1.19		A

Data correspond to the mean ± SD of five independent replicates. Means followed by different letters indicate significant differences among the varieties within each storage time (lowercase letters) and among storage times within variety (capital letters) based on Tukey-Kramer's test at $p < 0.05$.

Note: TSS = Total Soluble Solids (°Brix); TA = Titratable Acidity (as citric acid, g L-1 juice); LYC = Lycopene (mg 100 g^{-1} fresh fruit); T0 = harvest; T10 = ten days; T20 = twenty days; T30 = thirty days.

2.3.4 Texture analysis

The four parameters evaluated for the penetration test are presented in Table 8 for the turning group and in Table 9 for the red-ripe group.

For the turning group, we found interesting differences among varieties within storage time and within varieties among storage times. In particular, in EX1 both the force required to puncture the tomatoes skin (*Fp*) and the work necessary to reach the breaking point (*Wp*) of the commercial variety Camone at harvest were almost double than that required for the landraces Tamatta kaki and Tamatta cor'e boi (Tab. 8). This divergence slightly decreased during the storage with no significant differences among storage times while the landrace Tamatta cor'e boi emerged as the one with the significantly lowest values for both *Fp* and *Wp* at harvest and T10.A similar trend was observed for the firmness (*Ep*) for which the commercial variety Camone showed a value (*Ep* = 1.83 N mm^{-1}) significantly higher than those observed for the landraces Tamatta kaki (*Ep* = 1.18 N mm^{-1}) and Tamatta cor'e boi (*Ep* = 1.01 N mm^{-1}). The *Ep* decreased during the storage and no significant differences were registered among varieties after T10 (Tab. 8). The fruit deformation before the skin rupture (*Dp*) increased during the storage times with no significant difference among varieties at each storage time and within varieties, except for the landrace Tamatta kaki that showed a value of 0.69 mm at harvest and a final value of 1.18 mm at T20 (Tab. 8).

In EX2, the texture evaluation lead to results similar to those observed in EX1. Indeed, the commercial variety Camone exhibited the highest values at harvest for *Fp, Dp* and *Wp,* with decreasing values during the storage and no significant differences among varieties at T10 except for the *Fp* parameter (Tab. 8).

Table 8: Texture parameters as registered within the turning group in both experiments.

Turning Group												
EX1												
Varieties	**Fp**				**Dp**				**Wp**			
T0												
Tamatta kaki	0.82 ±	0.12	b	A	0.69 ±	0.05	a	B	0.27 ±	0.04	b	A
Tamatta cor'e boi	0.63 ±	0.06	b	A	0.62 ±	0.01	a	A	0.19 ±	0.03	b	A
Camone	1.35 ±	0.18	a	A	0.73 ±	0.08	a	A	0.46 ±	0.10	a	A
T10												
Tamatta kaki	0.68 ±	0.07	b	A	0.92 ±	0.22	a	AB	0.30 ±	0.06	ab	A
Tamatta cor'e boi	0.48 ±	0.06	c	A	0.80 ±	0.29	a	A	0.19 ±	0.09	b	A
Camone	1.05 ±	0.07	a	A	1.13 ±	0.28	a	A	0.53 ±	0.12	a	A
T20												
Tamatta kaki	0.67 ±	0.15	a	A	1.18 ±	0.18	a	A	0.38 ±	0.12	a	A
Tamatta cor'e boi	0.53 ±	0.15	a	A	0.91 ±	0.25	a	A	0.24 ±	0.14	a	A
Camone	-				-				-			
T30												
Tamatta kaki	-				-				-			
Tamatta cor'e boi	-				-				-			
Camone	-				-				-			
EX2												
Varieties	**Fp**				**Dp**				**Wp**			
T0												
Tamatticasa tundas a siccu	0.79 ±	0.09	b	A	0.54 ±	0.10	b	A	0.20 ±	0.05	b	A
Tamatta kaki	0.83 ±	0.12	b	A	0.52 ±	0.05	b	A	0.21 ±	0.04	b	A
Tamatta cor'e boi	0.91 ±	0.06	ab	A	0.67 ±	0.01	ab	A	0.28 ±	0.03	b	A
Camone	1.22 ±	0.18	a	A	0.95 ±	0.08	a	A	0.53 ±	0.10	a	A
T10												
Tamatticasa tundas a siccu	0.49 ±	0.19	b	A	0.97 ±	0.38	a	A	0.24 ±	0.16	a	A
Tamatta kaki	0.52 ±	0.07	b	A	0.69 ±	0.22	a	A	0.17 ±	0.06	a	A
Tamatta cor'e boi	0.44 ±	0.06	b	B	0.85 ±	0.29	a	A	0.18 ±	0.09	a	B
Camone	0.85 ±	0.07	a	B	0.86 ±	0.28	a	A	0.37 ±	0.12	a	A
T20												
Tamatticasa tundas a siccu	-				-				-			
Tamatta kaki	-				-				-			
Tamatta cor'e boi	-				-				-			
Camone	-				-				-			
T30												
Tamatticasa tundas a siccu	-				-				-			
Tamatta kaki	-				-				-			
Tamatta cor'e boi	-				-				-			
Camone	-				-				-			

Data correspond to the mean ± SD of five independent replicates. Means followed by different letters indicate significant differences among the varieties within each storage time (lowercase letters) and among storage times within variety (capital letters) based on Tukey-Kramer's test at $p < 0.05$.

Note: Fp= force required to puncture tomato skin (N); Dp= fruit deformation before skin rupture (mm); Wp= mechanical work necessary to reach the breaking point (N.mm); Ep= stiffness ($N\ mm^{-1}$).

T0 = harvest; T10 = ten days; T20 = twenty days; T30 = thirty days

On the other hand, significantly different results were registered among storage times within varieties for *Fp* and *Wp* which evidenced a declining

performance of the landrace Tamatta cor'e boi. Significant differences among varieties for the *Ep* parameter at harvest were not detect, but poorer performances were detected overtime with the commercial variety Camone emerging again as the one with the highest values (*Ep* = 0.99 N mm^{-1}) and the landrace Tamatta cor'e boi as one with the lowest values (*Ep*= 0.52 N mm^{-1}; Tab. 8).

Regarding the red-ripe group in EX1, *Fp*, *Dp* and *Wp*, did not reveal significant differences among storage times (Tab. 9), with the only exception of the commercial variety Datterino that showed at T20 its worst *Wp* value (0.33 N.mm) that was nonetheless quite valuable when compared to the other varieties. Among varieties within storage times, the landrace Arraccadas showed the lowest *Fp* at T10 (0.56 N; Tab. 9) but a *Wp* parameter similar to the commercial variety Datterino (Tab. 9); the landrace Lorigheddas de appiccai showed the poorest performance at T10 (*Wp* = 0.71 N.mm). The *Ep* parameter evidenced both significant differences among varieties within storage time and within varieties among storage times (Tab. 9). The commercial variety Datterino was noted to be the firmest at each storage time, with an initial value of 1.25 N mm^{-1} and a final value of 0.97 N mm^{-1} after 20 days of storage.

In EX2, a more complex pattern emerged. For *Fp* no significant differences were seen among varieties within storage time except at T20 when the landrace Tamatta groga de appiccai showed its poorest performance (*Fp*= 0.51 N; Tab. 9). Besides, the only difference within varieties among storage time was seen for the commercial variety Datterino that progressively decreased its skin compactness from harvest to T30 (Tab. 9). The *Dp* and *Wp* parameters increased notably with the advancing of the storage time, showing significant differences among genotypes at each storage times and within varieties among storage times. For example, the landrace Tamatta groga de

appiccai and the commercial variety Datterino recorded the lowest *Dp* values at each storage time, result confirmed by the *Wp* parameter's values (Tab. 9).

Table 9: Texture parameters as registered within the red-ripe group in both experiments.

Red-ripe Group																
EX1																
Varieties	**Fp**				**Dp**				**Wp**				**Ep**			
T0																
Arracadas	0.75 ±	0.14	a	A	0.77 ±	0.13	a	A	0.27 ±	0.10	a	A	0.97 ±	0.06	b	A
Lorigheddas de appiccai	0.84 ±	0.07	a	A	1.04 ±	0.25	a	A	0.43 ±	0.12	a	A	0.83 ±	0.12	b	A
Datterino	0.80 ±	0.01	a	A	0.64 ±	0.03	a	A	0.25 ±	0.02	a	B	1.25 ±	0.07	a	A
T10																
Arracadas	0.56 ±	0.04	b	A	1.28 ±	0.30	a	A	0.33 ±	0.07	b	A	0.46 ±	0.12	b	B
Lorigheddas de appiccai	0.76 ±	0.05	a	A	2.02 ±	0.67	a	A	0.71 ±	0.19	a	A	0.41 ±	0.15	b	B
Datterino	0.85 ±	0.08	a	A	0.90 ±	0.28	a	A	0.30 ±	0.04	b	AB	1.01 ±	0.31	a	A
T20																
Arracadas	-				-				-				-			
Lorigheddas de appiccai	-				-				-				-			
Datterino	0.83 ±	0.06		A	0.85 ±	0.03		A	0.33 ±	0.04		A	0.97 ±	0.06		A
T30																
Arracadas	-				-				-				-			
Lorigheddas de appiccai	-				-				-				-			
Datterino	-				-				-				-			
EX2																
Varieties	**Fp**				**Dp**				**Wp**				**Ep**			
T0																
Arracadas	0.61 ±	0.08	a	A	0.67 ±	0.06	ab	B	0.20 ±	0.03	a	B	0.91 ±	0.13	bc	A
Lorigheddas de appiccai	0.66 ±	0.08	a	A	0.79 ±	0.12	a	B	0.23 ±	0.06	a	B	0.80 ±	0.06	c	A
Tamatta groga de appiccai	0.66 ±	0.19	a	A	0.40 ±	0.09	c	B	0.13 ±	0.06	a	A	1.64 ±	0.12	a	A
Datterino	0.57 ±	0.04	a	B	0.52 ±	0.07	bc	C	0.15 ±	0.04	a	C	1.12 ±	0.07	b	A
T10																
Arracadas	0.64 ±	0.07	a	A	1.26 ±	0.02	a	A	0.37 ±	0.05	a	AB	0.51 ±	0.06	b	B
Lorigheddas de appiccai	0.70 ±	0.08	a	A	1.19 ±	0.18	a	A	0.41 ±	0.07	a	A	0.60 ±	0.09	b	B
Tamatta groga de appiccai	0.61 ±	0.08	a	A	0.59 ±	0.12	b	AB	0.18 ±	0.05	b	A	1.04 ±	0.09	a	B
Datterino	0.68 ±	0.06	a	AB	0.58 ±	0.05	b	C	0.20 ±	0.03	b	BC	1.17 ±	0.02	a	A
T20																
Arracadas	0.60 ±	0.11	ab	A	1.47 ±	0.15	a	A	0.43 ±	0.13	a	B	0.41 ±	0.03	c	B
Lorigheddas de appiccai	-				-				-				-			
Tamatta groga de appiccai	0.51 ±	0.03	b	A	0.79 ±	0.09	b	A	0.19 ±	0.03	b	A	0.65 ±	0.06	b	C
Datterino	0.74 ±	0.06	a	A	0.78 ±	0.08	b	B	0.29 ±	0.05	ab	AB	0.94 ±	0.03	a	B
T30																
Arracadas	-				-				-				-			
Lorigheddas de appiccai	-				-				-				-			
Tamatta groga de appiccai	-				-				-				-			
Datterino	0.71 ±	0.07		AB	1.01 ±	0.07		A	0.36 ±	0.06		A	0.70 ±	0.02		C

Data correspond to the mean ± SD of five independent replicates. Means followed by different letters indicate significant differences among the varieties within each storage time (lowercase letters) and among storage times within variety (capital letters) based on Tukey-Kramer's test at $p < 0.05$.

Note: Fp= force required to puncture tomato skin (N); Dp= fruit deformation before skin rupture (mm); Wp= mechanical work necessary to reach the breaking point (N.mm); Ep= stiffness ($N\ mm^{-1}$).

T0 = harvest; T10 = ten days; T20 = twenty days; T30 = thirty days

In EX2, the firmest varieties (*Ep* values) were the landrace Tamatta groga de appiccai and the commercial variety Datterino at each storage time, with respective values of 1.64 N mm^{-1} and 1.12 N mm^{-1} at harvest, and 0.65 N mm^{-1} and 0.94 N mm^{-1},at T20 (Tab. 9).

2.3.5 Principal component analysis

Principal component analysis (PCA) of all the parameters indicate that the first three principal components (PCs) cumulatively explain nearly the 91% of the total variation in both groups (Turning and Red-ripe) in EX1 and the 86% of the total variation in both groups in EX2 (Tab. 10).

Table 10: Total variance explained by the first three PCs and their eigenvalues in EX1 and EX2 and in both groups among all the accessions and storage times.

Turning Group							
EX1				**EX2**			
Component	**Total**	**% of variance**	**Cum %**	**Component**	**Total**	**% of variance**	**Cum %**
1	6.06	46.65	46.65	1	6.63	51.04	51.04
2	4.92	37.86	84.51	2	3.35	25.81	76.84
3	0.96	7.35	91.86	3	1.22	9.41	86.25
Red-ripe Group							
EX1				**EX2**			
Component	**Total**	**% of variance**	**Cum %**	**Component**	**Total**	**% of variance**	**Cum %**
1	6.64	51.07	51.07	1	6.27	48.26	48.26
2	3.63	27.93	78.99	2	3.47	26.67	74.93
3	1.57	12.04	91.03	3	1.42	10.96	85.89

In detail, in the turning group during EX1, the PC1 captured about the 47% of the total variation (Tab. 10) and showed high loadings for all parameters, except for *WL, VQ, L*, H°, Ep,* and *LYC* (Tab. 11)*;* the PC2 explained around 38% of the total variation (Tab. 10) and was significantly correlated to *WL, VQ, L*, a*, H°,Dp, Ep, LYC* (Tab. 11). The PC3 explained an additional 7% of the total variation (Tab. 10). In EX2, the PC1 captured about the 51% of the

total variation (Tab. 10) and showed high loadings for *WL, VQ, L*, a*, b*, H°, Dp, Ep, LYC* (Tab. 11); the PC2 explained around 26% of the total variation (Tab. 10) and was correlated to *C, Fp, Wp, TSS, TA* (Tab. 11). The PC3 explained around 9% of total variation (Tab. 10).

Table 11: Correlation between the first two PCs and all parameters among all accessions for both groups and trials.

Parameters[a]	Turning group				Red-ripe group			
	EX1		EX2		EX1		EX2	
	Component		Component		Component		Component	
	1	2	1	2	1	2	1	2
WL	0.55	-0.82	-0.86	0.10	-0.82	0.42	-0.73	0.33
VQ	-0.09	0.84	0.94	0.21	0.75	-0.45	0.86	0.05
L*	-0.55	0.69	0.90	-0.28	0.25	-0.81	0.61	-0.66
a*	-0.68	-0.68	-0.84	-0.38	0.83	0.19	0.45	0.77
b*	-0.76	0.54	0.77	-0.29	0.98	0.03	0.90	-0.34
C	-0.94	-0.12	0.18	-0.58	0.95	0.12	0.93	0.22
H°	0.21	0.95	0.93	0.21	0.43	-0.20	0.30	-0.86
Fp	0.81	0.51	0.51	0.76	0.54	0.52	0.36	0.39
Dp	0.59	-0.79	-0.87	0.30	-0.78	0.41	-0.94	0.22
Wp	0.92	-0.30	-0.35	0.84	-0.64	0.49	-0.78	0.38
Ep	0.31	0.92	0.93	0.23	0.97	-0.06	0.92	-0.20
pH	-0.92	-0.11	-0.23	-0.41	0.10	0.89	0.30	0.68
TSS	0.73	-0.05	-0.19	0.85	0.64	0.71	0.54	0.72
TA	0.82	0.12	0.12	0.76	-0.77	-0.10	0.76	0.32
Lyc	-0.57	-0.73	-0.87	0.12	0.64	0.73	0.42	0.81

[a]WL = weight loss (%); VQ = visual quality; L* = lightness; a* = redness-greenness; b* = yellowness and blueness; C = chroma; H° = hue angle; Fp= force required to puncture tomato skin (N); Dp= fruit deformation before skin rupture (mm); Wp= mechanical work necessary to reach the breaking point (N.mm); Ep= stiffness (N mm^{-1}); TSS = Total Soluble Solids (%); TA = Titratable Acidity (as citric acid, g L^{-1} juice); LYC = Lycopene (mg 100 g^{-1} fresh fruit).

When considering the red-ripe group in EX1, the PC1 captured about the 51% of the total variation (Tab. 10) and showed high loadings for all the parameters, except for *L*, H°, Fp, pH* (Tab. 11); the PC2 explained around 28% of the total variation(Tab. 10) and was correlated to *L*, pH, TSS and LYC* (Tab. 11). The PC3 explained around 12% of total variation (Tab. 10). In EX2, the PC1 captured about the 48% of the total variation (Tab. 10) and showed high

loadings for all the parameters, except for *a*, H°, Fp, pH* and *LYC* (Tab. 11); the PC2 explained around 27% of the total variation (Tab. 10) and was correlated to *L*, a*, H°, pH, TSS* and *LYC* (Tab. 11). The PC3 explained around 11% of the total variation (Tab. 10).

The first two principal components are plotted in Figures 5 and 6 for each harvest groups and year.

Regarding the turning group, the PCA allowed to distinctly separate the four storage times in both years highlighting the wide diversity among genotype in the different quality traits (Fig. 5).

In EX1 and EX2, PCA results evidenced very similar patterns in the distributions of the accessions also relatively to the loadings of the different parameters on the PCs. The main emerging difference is among the commercial variety Camone and the landraces Tamatta kaki and Tamatta cor'e boi. These latters, tend to group together at each storage time being usually characterized by the lowest *FP* and *Wp* and the highest *C*; in addition they were characterized by lowest values of *TSS* and *TA* (Fig. 5 A and C). The commercial variety Camone showed almost opposite characteristics (Fig. 5 A and C). An intermediate position was observed for the landrace Tamatticasa tundas a siccu, only present in EX2. The results were also useful to differentiate the accession among storage times, especially evident for T0 in respect to the others; this was mainly due to a loss of *VQ*, *Ep* and *H°* during the storage (Fig. 5 B and D).

Concerning the red-ripe group, the result showed that the commercial variety Datterino is distinctly differentiated from landraces for all parameters at each storage time in both experiments (Fig. 6 A and C). On the contrary the landraces grouped together at each storage time being usually characterized by the lowest *VQ*, *Ep* and *TSS* (Fig. 6 A and C).

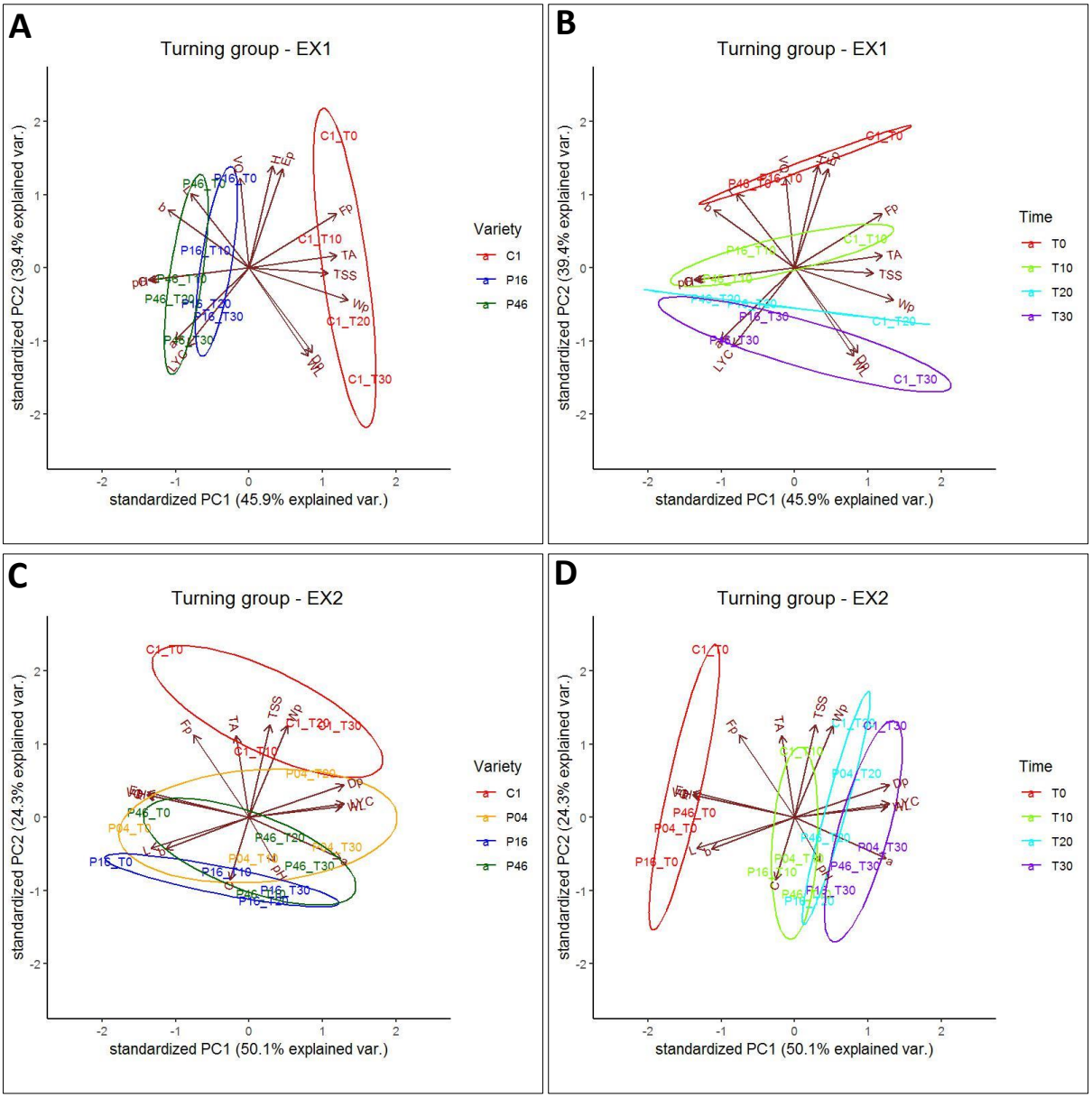


Figure 5: Scatter and loading (variables) plots of the first and second component obtained by the principal component analysis (PCA) based on 14 parameters within turning group in the EX1 (A, B) and EX2 (B, C). Different colors refer to different varieties (A, C) and different storage time (B, D).

Note: C1 = Camone; P04 = Tamatticasa tundas a siccu; P16 = Tamatta kaki; P46 = Tamatta cor'e boi; T0 = Harvest; T10 = tend days of storage; T20 = twenty days of storage; T30: thirty days of storage. WL = weight loss (%); VQ = visual quality; L* = lightness; a* = redness-greenness; b* = yellowness and blueness; C = chroma; H° = hue angle; Fp = force required to puncture tomato skin (N); Dp = fruit deformation before skin rupture (mm); Wp= mechanical work necessary to reach the breaking point (N.mm); Ep= stiffness (N mm^{-1}); TSS = Total Soluble Solids (%); TA = Titratable Acidity (as citric acid, g L^{-1} juice); LYC = Lycopene (mg 100 g^{-1} fresh fruit).

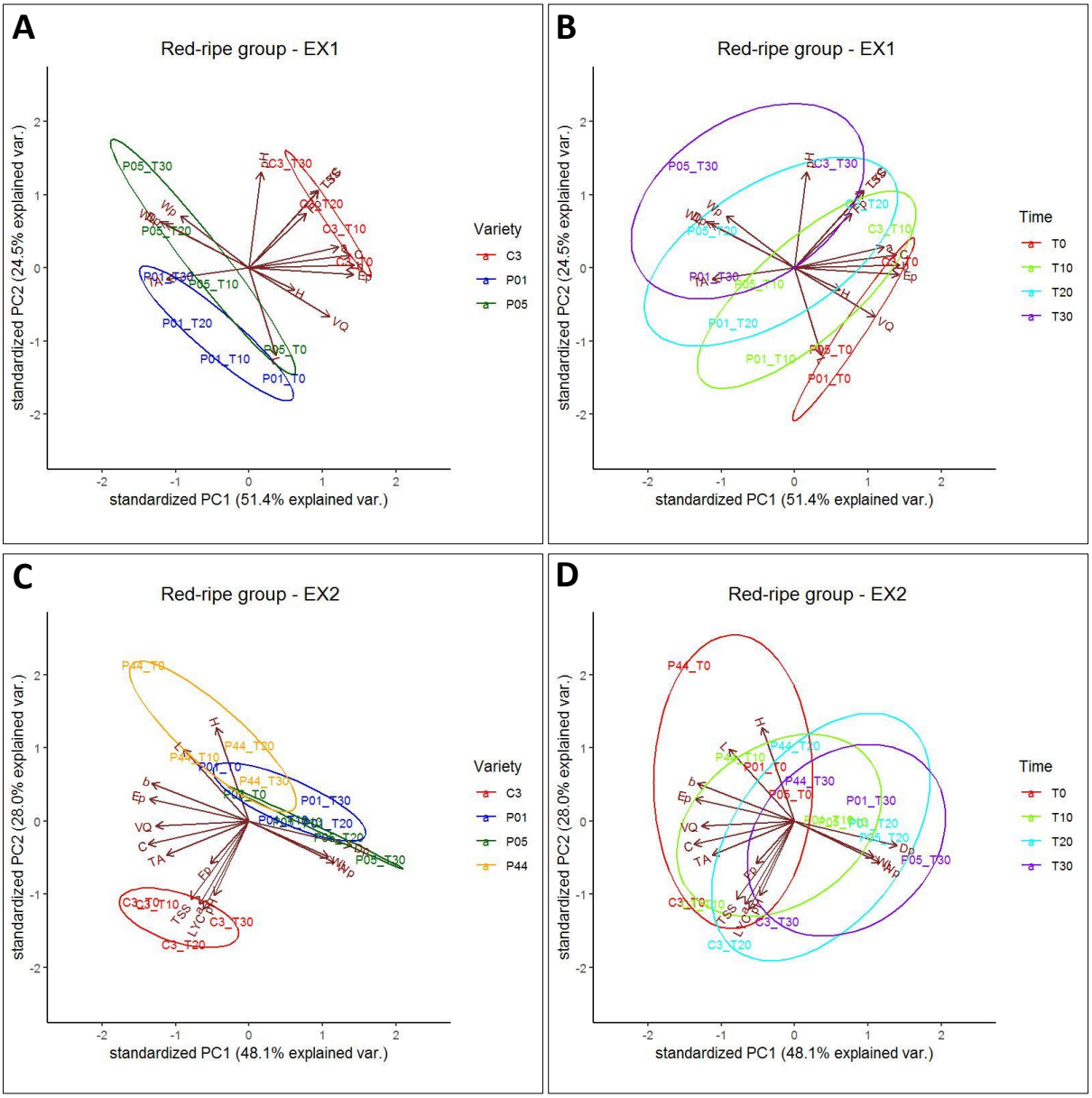


Figure 6: Scatter and loading (variables) plots of the first and second component obtained by the principal component analysis (PCA) based on 14 parameters within red-ripe group in the EX1 (A, B) and EX2 (B, C). Different colors refer to different varieties (A, C) and different storage time (B, D).

Note: C3 = Datterino; P01 = Arracadas; P05 = Lorigheddas de appiccai; P44 = Tamatta groga de appiccai; T0 = Harvest; T10 = tend days of storage; T20 = twenty days of storage; T30: thirty days of storage.

WL = weight loss (%); VQ = visual quality; L* = lightness; a* = redness-greenness; b* = yellowness and blueness; C = chroma; H° = hue angle; Fp = force required to puncture tomato skin (N); Dp = fruit deformation before skin rupture (mm); Wp= mechanical work necessary to reach the breaking point (N.mm); Ep= stiffness (N mm^{-1}); TSS = Total Soluble Solids (%); TA = Titratable Acidity (as citric acid, g L^{-1} juice); LYC = Lycopene (mg 100 g^{-1} fresh fruit).

In the EX2, it is interesting to note that the landrace Tamatta groga de appiccai, emerged as peculiar in respect to the other landraces and the commercial variety especially at T0, being characterized by the highest *L* and *H*. In EX1 and EX2, PCA results evidenced similar patterns for the storage times also highlighting no definite differentiations when passing from T0 to T30, except in EX1 where T0 was separated from the other (Fig. 6 B and D).

2.4 Discussion

Changes in overall appearance, color, texture and chemical components have been considered in the varieties object of this study, to evaluate the response to 30 days of storage with two experiment in two consecutive years (2017-2018 and 2018-2019, EX1 and EX2, respectively). The difference between the two experiments were significant for most of the traits in both turning and red-ripe groups. This could be the result of the different response of the varieties to the different meteorological conditions occurred in the two years. For example, during the EX1 the sum of the precipitations for the period from September to December was 162 mm, while during the EX2 the precipitation in the same period were 490 mm. This might have caused higher humidity within the greenhouse during the EX2 thus promoting the presence of various pathogens, such as *Phytophthora infestans* and *Botrytis cinerea.*

As shown in the results, none of the landraces in both EX1 and EX2 reached 30 days of storage in both groups, where most fruits were un-marketable. In general, the overall appearance for all the accessions declined rapidly, due to the appearance of visual defects in the fruit surface (e.g. shriveling, bruising, pitting) and to a high percentage of rotten fruits.

Among the landraces, the quick loss of freshness and appearance decline for Tamatticasa tundas a siccu and Lorigheddas de appiccai, revealed a faster

decay, probably due to a different genetic background when compared to other varieties, especially the Datterino. Indeed, the commercial varieties are usually selected for extended shelf-life (Mubarok et al., 2015; Uluisik et al., 2016) while the two landraces above cited were selected by local farmers for different traits, as suggested by their local names. Specifically, Tamatticasa tundas a siccu literally mean “Round tomato grown in low water conditions“ while the Lorigheddas the appiccai (literally “Earrings to hang up”) were mainly used as dried tomatoes after a long storage in dry and dark conditions tied up in circles and hung up from the ceiling of a pantry. Among the commercial varieties, Datterino showed the most extended shelf-life (20 days in EX1 and 30 days in EX2), while the shelf-life of Camone ended up after 10 days of storage in both experiments, in particular in EX1 was lower than the shelf-life of landraces.

A significant increase of weight loss was observed during the storage time in both experiments and groups. Minor differences emerged between landraces and commercial varieties, indicating a uniform pattern for this trait. Similar results were observed by Javanmardi and Kubota (2006), which attributed the main cause of weight loss in fruits stored at room temperature to the increased transpiration rate.

Color is an important quality attribute of tomato fruits which influences consumer purchase and, in the present study, interesting differences emerged. In the turning group, a general decrease of lightness (L^*) and yellowness (b^*) during the storage and a consequent increase of redness (a^*) were observed in both EX1 and EX2. These changes were also confirmed by the decrease of the hue angle ($H°$) indicating an increase of the reddish color of the fruit. Modifications of fruit color across the ripening stages are a consequence of the increasing maturation. The fruits harvested at the turning stage are predominantly green and then become progressively red during the storage (Batu, 2004). Thus, the decrease of L^* reflects the darkening of the

fruit color, which is, in turn, correlated with the carotenoid synthesis and, in particular, the increase in the *a** values is a consequence of the synthesis of lycopene and the degradation of chlorophyll (Arias et al., 2000). Overall, no significant variations were detected in the color of the red-ripe group during the storage in the EX1. This was mainly due to the fact that, at this maturity stage, the fruits were almost completely red at harvest (Batu, 2004). On the contrary, in the EX2 some variations were detected in the color parameters. For example, color changes in the commercial variety Datterino were not visually detectable but objectively confirmed by the decrease in *C* and *a** values at T30, probably due to the over ripening of the fruits (Batu, 2004). An increase of the redness of the fruits was also detected in EX2 in the landrace Tamatta groga de appiccai, which is a peculiar local variety characterized by an orange skin and red flesh at full ripe stage, characteristic confirmed by the highest hue angle values during the conservation (*H*° = 90° indicate pure yellow color). This local variety is an interesting product to be directly used in the markets, because it combines an attractive color with the benefits of the carotenoids. In general, interesting correlations were found among colors parameters and lycopene content of the fruits: in the turning group, the *a** values were positively correlated to the *LYC* (0.89 and 0.74 for EX1 and EX2, respectively) and negatively correlated to the *H*° (-0.79 and -0.83 for EX1 and EX2, respectively), indicating that modifications of fruit color are associated to the increasing LYC accumulation during the ripening process; also, in the red-ripe group the *a** and *C* values were positively correlated with the *LYC* content (*a** = 0.66 and 0.81 for EX1 and EX2, respectively; *C* = 0.69 and 0.57 for EX1 and EX2, respectively). These results are in agreement with previous finding by Arias et al. (2000), highlighting the nutritional value of tomato fruits and the possible benefits deriving from its consumption (Rao and Rao, 2007, Adalid et al., 2010).

Key parameters that contribute to the flavor and the nutritional value of tomato, as pH, total soluble solids (*TSS*), titratable acidity (*TA*) and lycopene (*LYC*) content, were also evaluated. The *pH* value of the tomato fruits increased with small and inconsistent differences among storage times in both experiments and groups, with the exception of the landraces Tamatta cor'e boi in EX1 and the commercial variety Datterino in EX2 which showed increasing acidity levels during the storage. The increase of *pH* values in these two varieties was probably due to the progress of the ripening (Teka, 2013). In parallel, an inconsistent decrease in *TA* was observed in both groups, due to a loss of citric acid which confirmed that acid concentrations in the fruit decline with maturity (Teka, 2013). All varieties from both groups did not show *pH* values above 4.4, the maximum desirable limit for a good taste (Gomez et al., 2001), the only exception was the commercial variety Datterino at T30 that showed a *pH* = 4.55. This variety also showed the highest pH values, with often no significant differences among the varieties, at each storage time indicating its lower acid content. Soluble solids are an indicator of fruit sweetness which usually increase during the maturity process, due to a degradation of polysaccharides. *TSS* values were quite stable during the storage in both experiments showing no significant changes in both groups. The same trend has been reported by Javanmardi and Kubota (2006), who reported modest changes in the ratio of glucose/fructose and organic acids during the storage. The landrace Arracadas showed in both EX1 and EX2 the lowest *TSS* content, characteristic that can be appreciated by people that need to reduce the blood insulin level (Renna et al., 2018). As expected, an increase in the *LYC* content, which are associated with the ripening process, was observed in the fruits of the turning group in both experiments (Jovanmardi and Kubota, 2006; Arias et al., 2000). On the contrary, in the red-ripe stage the *LYC* content was quite stable during the storage with slight but not significant increase, indicating that the synthesis of lycopene occurs up to the ripe stage, with no further synthesis (Arias et al., 2000). The common lycopene content in tomatoes is

usually around 6 mg 100 gr^{-1} of fresh fruit (Adalid et al., 2010). In the present study, for example, the landraces of the red-ripe group Arracadas and Lorigheddas de appiccai at T0 in EX1 and the landraces Tamatticasa tundas a siccu and Tamatta kaki at T10 in both experiments, showed *LYC* contents equal or higher to this average content. This suggests that these landraces could be considered interesting materials to be used in breeding programmes aimed to develop new improved cultivars with increased lycopene content (Adalid et al., 2010).

Texture of fleshy fruits and vegetables is another key factor affecting marketability and consumers' acceptance. Four parameters were evaluated, but among all the *Ep* is the most important and indicative of the softening levels of the fruit. In both turning and red-ripe groups, as well as in both experiments, a decrease of *Ep* during the storage were observed, due to an increase of ripeness or an over-ripeness of the fruits. At harvest, the varieties of the turning group were firmer than those of the red-ripe group, thus confirming that the level of softening of the fruit is affected by the maturity stage at harvest time (Teka, 2013). More in detail, in the turning group the commercial variety Camone showed the highest *Ep* values at each storage time, followed by the landrace Tamatta kaki in both experiments, with no significant differences among varieties after T10 in EX1. Also, in the red-ripe group the commercial variety Datterino was noted to be the firmest at each storage time in EX1, while at T0 in EX2 the landrace Tamatta groga de appiccai recorded the highest *Ep* values and at T10 did not show significant difference from the commercial variety Datterino. All these results confirmed that the fruit softening is related to the different susceptibility of tomato fruit to storage and the decrease in firmness may be related to the different polysaccharides degradation pathway (Teka, 2013). In both turning and red-ripe group, as well as in both experiments, *Ep* was significantly negatively correlated to *WL* (-0.61 and -0.72 for EX1 and EX2, respectively, for the turning

group; -0.83 and -0.62 for EX1 and EX2, respectively, for the red-ripe group) and positively correlated to *VQ* (0.69 and 0.88 for EX1 and EX2, respectively, for the turning group; 0.78 for both EX1 and EX2 for the red-ripe group), confirming that water loss is one of the main causes of fruit deterioration (D'Aquino et al., 2016). These parameters, together with color, sugar and acids and their interaction, are considered as the most important parameters affecting consumers acceptance and their perception of quality (Causse et al., 2003; Serrano-Megías and López-Nicolás, 2006; D'Aquino et al., 2016).

Multivariate statistical analysis was also performed in order to evaluate simultaneously all quality characteristics of the different genotypes. This allowed to clearly differentiate local varieties from the commercial varieties used as a control in both groups and experiments. Also, the PCA allowed to distinctly separate the four storage times in the turning group in both experiments, especially highlighting gradually different tomato fruit quality characteristics (Teka, 2013). On the contrary, in the red-ripe group, significant changes in the storage times were not clearly evident while it was possible to detect a pronounced distance between the commercial variety Datterino and the landraces. These latest tended to group together at each storage time, except for the landrace Tamatta groga de appiccai in EX2 at T0. This result showed the deep differences that exist between the two varieties Datterino e Tamatta groga de appiccai, in particular for the color characteristics and the *LYC* content. In general, on the basis of all the analyzed data, both commercial varieties used as a control are different from all the landrace evaluated at each storage time, but among the landraces, some of them showed characteristics of particular interest, confirming the great potential of local varieties (Adalid et al., 2010)

The performance of all accessions in terms of response to the storage, nutritional composition and market-related quality attributes, could have been influenced by the variation in environmental conditions in which the

varieties were grown, such as light intensity, temperature and air humidity (Arah et al., 2015; Iglesias et al., 2015; Sánchez-González et al., 2015; Bertin and Génard, 2018). In particular, most of the aroma and health compounds accumulation, such as sugar and lycopene, are promoted by temperature above 10°C during the growth period and regulated by the spectral composition of the light (Bertin and Génard, 2018). Nevertheless, *pH*, *TSS* and *LYC* ranges found in these experiments, can be considered satisfactory in fresh tomatoes when compared to the levels usually observed in standard growing conditions (open field during summer season; *e.g*. TSS = 4.47 °Brix for mature green compared the *TSS* values found in the present research of 4.2 and 4.1 °Brix for the landraces Tamatta kaki and Tamatta cor'e boi, respectively, at T0 in EX1; Teka, 2013) or in similar environments (greenhouse during spring/summer season) with different management (hydroponically cultivation; *e.g. LYC* = 9.25 mg 100 g^{-1} for red maturity stage compared the *LYC* values found in the present research of 8.11 and 10.60 mg 100 g^{-1} for the landrace Lorigheddas de appiccai and the commercial variety Datterino, respectively, at T0 in EX1; Arias et al., 2000).Also, the crop management, such as irrigation and fertilization, can affect tomato quality (Bertin and Génard, 2018).

2.5 Conclusions

The aim of this study was to investigate a collection of Sardinian tomato landraces for parameters that play a determinant role to evaluate their response to conservation and, as a consequence, in consumers' acceptance. Indeed, important quality characteristics, that determine marketability, nutritional value and flavor of fruit, were determined during thirty days of storage.

This study allowed to trace out for the first time an evaluation of the response to storage and exhaustive quality characterization of the tomato Sardinian landraces. The data presented in this work highlight the potential of some local varieties, which will might be used in further genomic research as well as source of useful genes for future breeding programs. These accessions represent also a valuable material to promote their direct valorization in local markets, due to their distinctive traits that can satisfy special market needs. Also, the present results might help in promoting the *in-situ* conservation of these traditional tomato varieties by local farmers.

Future studies are required to fully understand the role of the season, environments and the pre- and post-harvest conditions (e. g. light and temperature) that may affect the quality characteristics of these local varieties.

As well, it would be of great interest to perform a sensory analysis by a consumer's panel (Serrano-Megías and López-Nicolás, 2006; D'Aquino et al., 2016). The perception of different attributes, such as taste intensity, sweetness, acidity and hardness, would be an interesting information that would allow to investigate the consumer preference and relate their perception with the objective detected characteristics of the local varieties.

CHAPTER 3

3 The value of agrobiodiversity: an analysis on consumers preferences

3.1 Introduction

Following the 1992 world summit held in Rio de Janeiro, different governments adopted the Convention on Biological Diversity (CBD, 1992) in which biodiversity is defined as the variability between all living organisms and the ecological complexes of which they are part of. Agricultural biodiversity, or agrobiodiversity, is a small component of biodiversity and its concept was introduced by FAO considering it as "the variety and variability of animals, plants and micro-organisms at the genetic, species and ecosystem levels that sustain the ecosystem structures, functions and processes in and around production systems".

Agrobiodiversity plays a key role in food improvement and nutrition security (Frison et al., 2011). The sectors of food, renewable primary products, industrial biomass and bioenergy plant production cannot prescind of agrobiodiversity (Schröder et al., 2007). But, over the last century, three-quarters of the genetic diversity found in agricultural crops have been lost (Schröder et al., 2007). This genetic erosion is still continuing leading to a constant loss of agrobiodiversity (Jackson et al., 2007). As example of this process, the cultivated tomato has less than 5% of the total genetic variability of the *Solanum lycopersicum* species (Miller and Tanksley, 1990).

The International Treaty on Plant Genetic Resources for Food and Agriculture, negotiated by FAO Commission in 1996, promote the conservation

of plants at risk genetic erosion through both *in-situ* (on farm) and *ex-situ* (Gene Banks) strategies. The treaty also engages parties for a sustainable use of the genetic resources and recognizes the role and rights of farmers in conserving, using and improving agricultural genetic resources. More recently, the importance of varieties at risk of genetic erosion is highlighted in the UE report "Agricultural Genetic Resources - from conservation to sustainable use" (2013), in which the conservation and commercialization of these products are encouraged, promoting the implementation of policies and programs and the use of marketing strategies to valorize agrobiodiversity (e.g. label or logo to indicate the added value of the product) (Oehen et al., 2018).

The use of genetic resources plays a key role in the preservation of agrobiodiversity and, among them, the landraces, i.e. locally adapted varieties, constitute the main source of variation in the cultivated species, thus justifying the increased interest in their utilization (Brush, 2000; McCouch, 2013). The ancient local varieties represent an important crop heritage and possess sensory characteristics that consumers esteem (Causse et al., 2003). The conservation of the landraces is nowadays widely achieved and along with this it will be of significant importance to increase the performance of some of the most productive crops and boost sustainable agriculture and environmental protection (Brush, 2000; McCouch, 2004). The use of landraces in local markets contribute in ensuring the sustainability of rural communities and satisfy consumer demands (Brugarolas et al., 2009). But, an issue with local varieties is their high market price, a direct consequence of production costs due both to lower productivity and reduced resistance to pathogens than commercial hybrids (Brugarolas et al., 2009). But, despite the higher prices, a wide sector of consumers is willing to buy these products, often characterized by high quality and better sensory characteristics (Balogh et al., 2016).

The successful conservation and utilization of biodiversity in agricultural requires new types of cooperation among researchers, breeders, agronomists, ecologists, and economists to identify and establish adequate assessment and valorization strategies (Jackson et al., 2007). Also, consumers and agro-food industries have an important function, as they determine the decisions across the supply chain and influence the production patterns (Bothelho et al., 2018).

Consumers decisions in food purchase are influenced by several factors, such as environmental, production, nutritional and qualitative concerns (Moser et al., 2011). Consumers perception of quality is influenced by intrinsic and extrinsic attributes of the products, some of which can be evaluated before the purchase (*e.g.* price, dimension, size, color) while others can be determined only after consumption (*e.g*. taste, flavor and convenience) (Moser et al., 2011; Oltman et al., 2014). Among all this characteristics, color, size and shape are determinant for the consumer purchase (Serrano-Megías and López-Nicolás, 2006; Kader, 2008; Causse et al., 2010). Nonetheless, recently some studies revealed the consumers concerns about standardized products and long food miles and energy dispersion in the supply chain, indicating an increased consumer distrust in global markets and a consequent increased attention versus the quality of the products and sustainability (Giampietri et al., 2018).

Indeed, consumer demand for niche products has significantly increased, especially for organic food, locally grown products and traditional food (Balogh et al., 2016, de-Magistris and Gracia, 2016; Annunziata and Vecchio, 2016; Skreli et al., 2017; Meyerdinget al., 2019;). Accordingly, studies on consumers preferences of traditional and local varieties started to emerge. For example, Botelho et al. (2018) investigated the consumers' preference for traditional varieties of apple in Portugal, demonstrating that consumers are actually willing to pay (WTP) for the feature "traditional variety". The aim of

the research of Rocchi et al. (2016) was the evaluation of agrobiodiversity and its role for the local community, focusing on the tomato landrace "Pomodoro di Mercatello" from Perugia, Italy. They found that the values that consumers were willing to pay for purchasing this landrace were higher than the price actually charged in the market, suggesting the possibility to adopt strategies for the valorization of these types of products. Brugarolas et al. (2009) determined that the prices that consumers would be willing to pay for local Spanish tomato varieties were high enough to amply compensate for the additional costs of their cultivation. They also investigated the influence of several attributes on willingness to pay for these products and results showed that consumers are willing to pay high premium prices for the traditional flavor of the tomato landraces (Brugarolas et al., 2009).

These studies represent an example of the importance to valorize the genetic resources and demonstrate that these products can be appreciated by consumers whereas farmers might produce local varieties without losing income. The purchasing attitude and consumers behavior have been widely explored, primarily using preference techniques such as Choice Experiments (CE) (Moser et al., 2011). This method allows to extend the knowledge about the value of agrobiodiversity assigned by consumers and design future programmes concerning the traditional varieties to encourage farmers to conserve and cultivate these valuable genetic resources.

In accordance with this premise, a CE analysis on 920 consumers was carried out in order to estimate their willingness to pay (WTP) for consuming local tomato varieties (landraces) rather than commercial varieties. More in detail, the aims of this work were to determine the potential value of this kind of products according to the preference of the consumers and to assess the importance (weight) that respondents assign on each of the attributes chosen to describe the good. Estimation of willingness to pay might allow to verify which attributes mostly contribute to describe consumers' preferences and if

the landraces' characteristics reflect their preferences. The results obtained with this research could be useful to plan strategies and programmes to support the cultivation of local tomato varieties and to develop regional and national markets adapt to acknowledge their characteristic.

3.2 Background

Tomato (*Solanum lycopersicum* L.) is an important horticultural crop grown and commercialized worldwide all-year-round. Over the past two decades, tomato production has almost doubled, as has the area dedicated to its cultivation (FAOSTAT, 2020). Obviously, the increase in tomato production corresponds to its greater consumption. In fact, in the Mediterranean countries the highest average consumption of tomato has reached values between 20 and 100 kg/person/year (FAOSTAT, 2020).

In Italy, the cultivation of tomatoes has a long tradition and our country is the first producer in Europe (FAOSTAT, 2020). The importance of this species is also confirmed by the precious agrobiodiversity heritage still present today, which can represent an important resource to be studied and valorized which could meet the increasing consumer demand. Indeed, Italy is one of the richest countries in ancient tomato varieties and some of them are recognized by marks such as the Protected Designation of Origin (PDO) and the Protected Geographical Indication (PGI) (Mallamace et al., 2014; Sacco et al., 2017; Renna et al., 2018). Despite the recognized importance of ancient local varieties, their direct use in cultivation is rare. A way to counterbalance this tendency could be to use them as typical local products in a circuit that enhances local cultures. This would also have a positive impact on the protection of their biological heritage, an enhancement of the rural

environment and the development of sustainable agriculture and agritourism (Ceccarelli et al., 2000).

3.3 Materials and Methods

3.3.1 The Choice Experiment

A Choice Experiment (CE) approach was used to estimate the expected value of the "local" attribute in tomato sold in retail. In other terms, CE was applied for understanding if a willingness by a part of the consumers exists to pay a "premium price" to purchase local varieties of tomato, how much this surplus eventually is, and, as a consequence, if sellers can apply it.

Stated preferences of consumer in demanding local varieties were evaluated on the basis of different tomato characteristics.

CE methods comes from the Lancaster's characteristics theory of value (Lancaster, 1966) and from random utility theory (Thurstone, 1927; Manski, 1977). The rationale underlined is that any good, marketable or not, can be described in terms of presence and intensity of its inherent attributes, price included. Attributes that would be demanded by the consumers, not the good itself. The CE involves a hypothetical scenario and the presentation of a choice set, where it is asked at the respondents to express a preference among different alternatives, one of which is a base case. The alternatives are described by various characteristics, known as attributes, and the price (Moser et al., 2011). This method allows to determine the relative importance of various attributes of compared products in consumers' choice process (Moser et al., 2011). First, attributes and their levels need to be identified; then experimental design theory is used for generating more profiles of the good in terms of presence and intensity of its attributes.

3.3.2 The experimental design

The study was carried out taking into account three different marketable and independent attributes for tomato and a price attribute (Table 1).

The first one was the "variety" attribute. It was defined in two levels: the first level corresponds to local tomato variety (*i.e.* landrace) while the second level corresponds to commercial variety, used as alternative of the investigated product (Tab. 1). The latter corresponds to a generic variety and any information about the variety is given to the final consumer.

Table 1: Attributes and their levels used in the choice experiment design.

Attributes	Levels
Variety	Local / Commercial
Integrity (defects on the surface of the fruit)	Absence / Presence
Standardization (fruit dimension uniformity)	Absence / Presence
Premium price (€)	0.00 / 0.10 / 0.20 / 0.50

The second attribute was the "integrity". It was also defined in two levels, referring to the presence or absence of defects on the surface of the fruit (Fig. 1 A and B). This attribute was introduced in order to evaluate the role played by a characteristic that, even though not typical, is often present in the local varieties of tomato.

The third attribute was the "standardization". It indicates the uniformity in the dimension of the fruits, and it is a characteristic typically present in modern commercial varieties. The two levels taken on by this variable were present or absent (Fig. 1 C and D).

In other terms, the variables "integrity" and "standardization" were introduced for better assessing the consumer preferences with respect to the "local" attribute. These are two product characteristics of fruits and

vegetables which can influence consumer choices. Although common, for this reason they were treated as independent variables, the presence of imperfections and the lack of standardization are sometimes associated in the local tomato and it is therefore needed to evaluate the preferences with respect to the single attribute (*i.e.* "local") within a likely market context.

Finally, a "premium price" attribute was introduced, *i.e.* this attribute reflects the incremental price that consumers are willing to pay for a given choice. In the light of the suggestions obtained after a submission of a pre-test to a sample of interviewers, we set the (marginal) prices at 0.00, 0.10, 0.20, 0.50 Euros (Tab. 1).

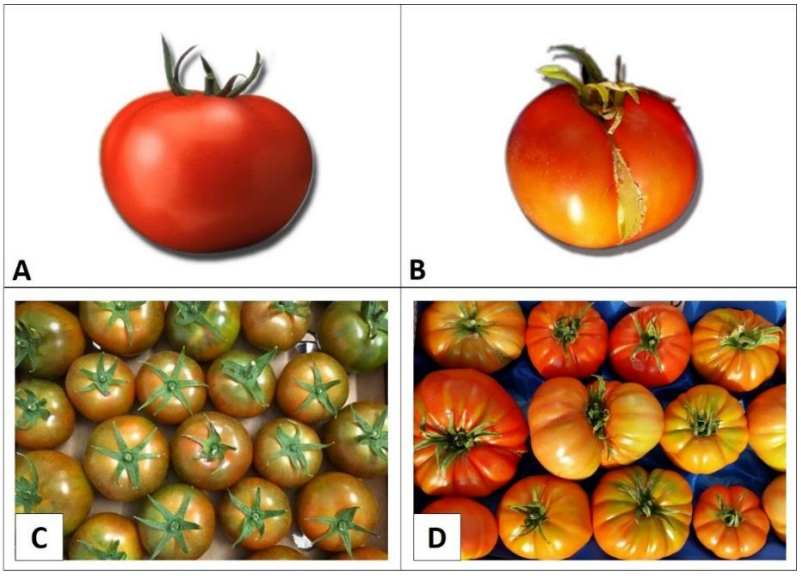


Figure 1: Representations of integrity and standardization attributes: intact fruit (A) and fruit with an imperfection on the surface (B) and standardized variety characterized by fruits with uniform shape and size (C) and non-standardized variety with very variable shape and size of fruits (D)

After a test on 50 interviews, the so-called pre-test, a random sample of 920 consumers throughout Italy were interviewed. The preliminary test was launched in order to verify the design, the comprehensibility of the content and the price attribute. The final questionnaire was submitted by an online

survey created with the LimeSurvey open source PHP web application software (http://limesurvey.org/) and it was open to participants for 10 weeks.

The survey was divided into five sections. The first one informed the participants about the relevance of the biodiversity preservation and the importance of the valorization and utilization of the genetic resources, allowing to illustrate the issue and to describe the characteristics of this kind of products. In the second section, a hypothetical agrobiodiversity programme focused on the conservation and valorization of local tomato varieties was presented to the participants. In here each consumer was invited to participate through the payment of a premium price when purchasing a local variety. Some instructions to fill in the survey were also given. The third section contained the cards with the choice options. In the fourth section, six questions were proposed to examine consumption preferences and general purchase behavior of participants (*e.g*. relevance attributed to local varieties, frequency of consumption and place of purchase). Finally, the fifth section contained some socioeconomic questions (gender, age, region of origin, education level and profession). The survey ended up with an open-ended question in order to collect suggestions, advices, clarifications, critics or anecdotes.

Specifically, in the section three we showed ten random cards for each respondent. Each card was composed by three different alternatives and each alternative showed a random combination of levels for each attribute. More precisely, 3 different options were represented in each card:

- the *status quo* represents the basic option and it was always showed in each card. It is defined by the level “commercial” for the attribute “variety”, “absence” for the attribute “integrity” and “presence” for the attribute “standardization”. The price level is equal to 0.00 Euros.

- Two different alternatives. They were different for the attributes of each level and with respect to the *status quo* (Fig. 2)

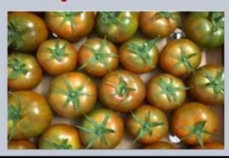
Option	Variety	Integrity	Standardization	Premium Price
1	commercial	absent 	absent 	0 €
2	local	absent 	present 	0,20 €
3	local	present 	present 	0,50 €

Figure 2: An example card of the choice experiment.

Each of the three alternatives reported in a card was randomly generated. Considering the numbers of levels (four) and the relative attributes (2 * 2 * 2 * 3), 18 of the 24 possible alternatives were generated.

Indeed, we excluded the alternatives where levels of attributes are equal to the *status quo* except for the price. These alternatives were excluded because not compatible with the rational behavior of a given consumer (any premium price would be paid if the good shows identical characteristics). Furthermore, the options where the only level that varies (with respect to the *status quo*) is "local" was excluded. This is a methodological choice due to the constraint of ignoring the only variance of the attribute that represents the focus of the study (*i.e.*, the cases where "local" varies and the other two do not). The underlined rationale is to ever collocate the "local" attribute in a complex and dynamic framework where it (eventually) change together with other tomato characteristics.

It means that 153 possible combinations of the 3 options (the *status quo* is a fixed option) can be obtained.

The ten cards were casually submitted to respondents, who were invited to express just one preference per card. The algorithm used to submit the cards was studied to allow that all of the 153 combinations were equally represented in the sample.

3.3.3 The adopted model

A Conditional Logit model was used to estimate the findings. This model allowed to estimate the probability of selecting a specific choice set by a part of the interviewed, distinguishing the choice attributes from each individual characteristic. Individual characteristics are used to take into account the heterogeneity of preferences:

$$(1) \qquad V_i = a_i + \sum \beta_k x_{ik} + \sum \delta_{hi} s_{hn}$$

where V_i is the deterministic (observable) component of the utility function, a_i is the alternative specific constant (ASC), β_k is the parameter vector associated to the attributes k ($k = 1….K$) of alternatives i noted (x_{ik}), and δ_{hi} is the parameter vector of the h ($h = 1…H$) characteristics of individual n (s_{hn}).

The *dummy* variable ASC indicates the utility associated with moving away from the basic alternative. In other terms, the value of the ASC suggests if an endowment effect (or a bias) associated to the basic alternative exists. In our case, it would indicate a possible endowment effect to the status quo option. Attributes (x_{ik}) are related to the attributes selected (Table 2). The first variable is equal to 1 in the case of “local” tomatoes and 0 in the case of “commercial” ones. The second dummy variable is equal to 1 when the product shows “integrity” and 0 when not. The third variable is equal to 1 in the case

of a "standardized" product and 0 *vice versa*. Finally, the fourth variable varies according to the four considered price levels.

Data were processed by N-logit software (Econometric Software, Inc., NY).

Table 2: Variables involved in the CE model.

Variable		Description
Constant	ASC	
Variety	X_1	0 = commercial; 1 = local
Integrity	X_2	0 = absence; 1 = presence
Standardization	X_3	0 = absence; 1 = presence
Price	P	0.00 0.10 0.20 0.50 Euros

3.4 Characteristics of the sample

Descriptive statistics about heterogeneity of the sample were performed: the sample of respondents showed an average age of 44 years old and it was characterized by 55.4% of females and 43.1% of males. Also, information about the level of education and the occupation were asked: 4.9% of respondents registered primary and middle educational degree or they did not answer; 24.6% of respondents declared to have secondary educational level and 70.5% were in possession of an academic graduation; about 60% of the participants in the survey were employees, teachers, researchers and students.

Consuming and purchasing behavior of the respondents were also investigated and the descriptive statistics are reported in Table 3.

Table 3: Consuming and purchasing behavior characteristics in the sample

Relevance attributed to local products in food consumptions	Respondents	
	n°	%
Very important	565	61.4
Important	307	33.4
Fairly important	28	3.0
Not important	5	0.5
Indifferent	7	0.8
No answer	8	0.9
Total	**920**	**100.0%**
Frequency in consuming fresh tomato		
Regular consumer	708	77.0
Not regular consumer	204	22.2
No answer	8	0.9
Total	**920**	**100.0%**
Preference in consuming tomato		
Salad	504	54.8
Sauce	124	13.5
Fresh	258	28.0
Other	26	2.8
No answer	8	0.9
Total	**920**	**100.0%**
Responsible for purchasing in the family		
Yes	697	75.8
No	215	23.4
No answer	8	0.9
Total	**920**	**100.0%**
Place of purchase		
Mass market retailers	170	18.5
Minimarket end self-service grocery store	103	11.2
Specialized stores	209	22.7
Neighborhood markets	227	24.7
Farm	167	18.2
e-commerce	3	0.3
Other	33	3.6
No answer	8	0.9
Total	**920**	**100.0%**

When asked to assign a value to the relevance of local products in food consumption, 61.4% of the interviewed assigned a very high relevance, 33.4% a high relevance, while a negligible percentage of respondent attributed a low or no importance at all to local products. The 77% of the respondents declared to be a regular consumer of fresh tomato; 54.8% of the sample preferred to consume tomato in salads, 28% as a fresh product and 13.5% as tomato sauce (Tab. 3). Most of the respondents declared to be the responsible for food purchasing in the family (75.8%). The 24% of the sample usually purchase fresh tomato in the neighborhood markets, 22.7% in specialized stores, 18.5% in mass market retailers, 18.2 % directly in the farm and 11.2% in minimarket or self-service grocery stores (Tab. 3).

3.5 Results

The CE model was carried out and findings are reported in Table 4. Total observations were equal to 27,600 (920 respondents * 10 cards * 3 alternatives).

Table 4: Estimated coefficients and values from CE application.

Parameter	Coeff.	s.e	Z	p-value		Value
Constant	-1.362	0.024	-56.750	0.000	***	
Local	1.976	0.035	56.457	0.000	***	€ 0.90
Integrity	0.983	0.033	29.788	0.000	***	€ 0.45
Standard	-0.368	0.033	-11.152	0.000	***	-€ 0.17
Price	-0.022	0.001	-22.000	0.000	***	
Log-Likelihood =	16,596.57	n: 27,600				

The Conditional Logit Model is based on the a priori assumption of the independence of irrelevant alternatives (IIA) property (Hanley et al., 1998). It means that the relative probabilities of two alternatives are unaffected by the introduction or removal of other alternatives (Ben-Akiva and Lerman, 1985). Therefore, the Hausman and McFadden (1984) test was applied to estimate the suitability of the Conditional Logit model to the data. Basically, the adopted model was compared with (from time to time) a restricted model in which an attribute is removed using the Generalized Log-likelihood ratio test.

It was estimated that the IIA property is not violated by the Conditional Logit Model adopted. Indeed, the values of the test statistics, given by the comparison between the coefficients estimated before and after removing one of the alternatives, are equal to 4.04, 4.66, and 5.17 for “local”, “integrity”, and “standardization”, respectively; each value is lower than the correspondent critical value of the chi-square distribution at 99.5% of confidence level. Therefore, the null hypothesis should not be rejected, implying that the Conditional Logit model well fits the data.

Concerning the model application, all estimated coefficients are statistically significant. The coefficient related to the price is negative as expected because the model is based on an inverse relationship between WTP and price. The ASC is statistically significant, meaning that a tendency to switch from the *status quo* alternative towards other alternatives would exist (*i.e.*, the *status quo* would not be the preferred alternative by final consumers).

The economic value related to each attribute is found calculating the inverse ratio of the attribute coefficient on the price coefficient:

$$(2) \quad WTP = - \beta_k / \beta_p$$

where WTP is the willingness to pay related to a specific attribute, β_k is the estimated coefficient related to this specific attribute, and β_p is the coefficient associated to the Price attribute. All calculated values were expressed in terms of Euros/Kg.

The "local" attribute shows the highest value, equal to 0.90 Euros. It means that consumers would be willing to pay a premium price of 0.90 Euros/Kg for purchasing local varieties of tomatoes. Considering the market price of tomatoes in Italy in 2019, it represents a remarkable additional value.

A 0.45 Euros/Kg value was estimated for "integrity". The positive sign indicates that consumers might pay a premium price for tomatoes that do not show imperfections (*e.g*. on the surface of the fruit). Despite this positive sign is an expected result, it suggests that willing to pay an additional value for local varieties (0.90 as before reported) would be resized considering that imperfections is a marketable aspect that often characterizes local varieties of tomatoes.

Finally, the "standardization" attribute shows a negative value (the magnitude is equal to 0.17 Euros/Kg). Basically, interviewers are willing to pay an extra price in case of varieties with very variable shape and size of fruits (*i.e.* not standardized fruits).

It must be underlined that attributes were handled as independent in this model, therefore estimated values can be added according to the level of the attribute. In other terms, according to the co-presence of these three attributes, presence/absence of each one, a premium price on the whole can be estimated. In the case of local tomato variety with not standardized and intact fruits, the premium price can reach up to 1.52 Euros/Kg. Conversely, for standardized supply and presence of imperfections, the premium price can decrease to 0.28 Euros/Kg.

3.6 Discussion

In this study, a choice experiment approach was carried out in order to investigate the willingness to pay for tomato local variety by consumers and determine their preferences among the different characteristics used to describe the product.

The present research allowed to determine the value that consumers attribute in general to the landraces, rather than for a specific variety. Indeed, the results showed that consumers are actually willing to pay a remarkable premium price for the feature “local variety” than for the “commercial” one. This result is in agreement to those found by Botelho et al. (2018), who sought to estimate the willingness to pay for the attribute “traditional variety” itself rather than determining the consumers’ willingness for a specific Portuguese apple variety.

Through this research, the value attributed to the local variety can be extended to a wider concept and considered as a representative value attributable to agrobiodiversity. The result obtained by the survey has therefore made possible to ascertain that consumers recognize a value (commercial and not) to agrobiodiversity, reveling their sensitivity, attention and knowledge about the subject and their willingness to contribute to its conservation and enhancement. As a proof of this feedback, the result can also validate by those found by both Rocchi et al. (2016) and Brugarolas et al. (2009) which are focused their studies on the Italian tomato landrace “Pomodoro di Marcatello” and the Spanish tomato landraces “Muchamiel” and “De la Pera”, respectively. Both studies show that respective local market consumers attribute a very positively value to these varieties and they are willing to pay very high premium prices higher than those charged in the market. However, these results are strictly connected to the territory and the

specific characteristics that these three local varieties possess and it is not possible to extend and attribute their value to the general feature “local variety”.

The results of the present study also revealed that consumers are willing to pay a premium price for the feature “integrity”, but not for standardized products. Thus, in a valorization prospective for local varieties, consumers might also pay the highest premium price for tomato varieties characterize by no-standardized and intact fruits, *i.e*. with very variable shape and size of fruits that do not show imperfections on the surface. Therefore, in the case of the co-presence of the three attributes local, integrity and no-standardization, the premium price that consumers are willing to pay can reach up the remarkable additional value, considering the market price of tomatoes in Italy during 2019, of 1.52 €/Kg. In this way, the cultivation of traditional varieties of tomato would be proposed as an economically interesting alternative for farmers. Any effects on the income of the farmers should be duly assessed and it was not the objective of this research, but the whole premium price could be high enough to encourage on-farm conservation, how suggested by Brugarolas et al. (2009). These authors determined that consumers would be willing to pay an average price of 2.72 €/Kg (81% surcharge) for the “Muchamiel” tomato landraces and 2.37 €/Kg (58% surcharge) for the “De la Pera” tomato landrace, prices that would well compensate the higher costs related to the cultivation of these local varieties. Indeed, the cultivation of tomato landraces involves higher crop management costs, due both to lower productivity and resistance to pathogens than commercial hybrids (Brugarolas et al., 2009). This is an important information for producers of local varieties because the additional price can sensitively vary according to the presence or absence of the integrity and standardization attributes. Indeed, for standardized products and presence of imperfections, the premium price can decrease to 0.28 €/Kg. Hence, the valorization of local varieties through their

cultivation also for marketable reasons might open up to quite different scenarios in terms of price and, therefore, of profitability for the farm and/or the entire supply chain. This latter finding is in accordance with that found by Botelho et al. (2018), in whose study the participants are willing to pay more for traditional Portuguese apple varieties than for non-traditional ones, but the premium price is not enough to encourage on-farm conservation.

However, altogether the results are promising, suggesting the possibility to adopt strategies and programmes for the valorization and promotion of these types of products through the support of their cultivation and development of regional and national markets adapted to acknowledge their characteristic. Indeed, the enhancement of marketing plans, the adoption of particular recognition marks, or the creation of specific brands could further stimulate the development of this niche market. For example, as reported by Balogh et al. (2016), quality certification was identified as one of the most important attributes in consumers decisions about traditional food products. As another example, solution could derive from the development of appropriate marketing plans based on the promotion of some pre-investigated nutraceutical proprieties or sustainable characteristics (*e.g*. short food miles) possessed by the product (de-Magistris and Gracia, 2016; Annunziata and Vecchio, 2016). Also, the promotion of environmental and socio-economic impacts derived from its preservation and cultivation, might be effective strategies that could contribute to a more profitable income for farmers (Sardaro et al., 2016). The commercial valorization of the varieties at risk of genetic erosion is also encouraged by international, national and regional organizations who intend to boost it through incentives and measures to support the farmers involved in landraces conservation programmes (Spataro and Negri, 2013).

3.7 Conclusions

The main objective of this study was to estimate the willingness to pay (WTP) of consumers for consuming local tomato varieties rather than commercial varieties and determine the potential value of this kind of products according to the preference of the consumers, which could increase the effectiveness and efficiency of conservation strategies.

In this regard, a choice experiment (CE) was carried out taking into account three different marketable and independent attributes to describe the good (local, integrity and standardization) and a price attribute. The results obtained, suggest that consumers appreciation and willingness to pay for a local variety is higher than for a commercial variety, especially if the fruits of the landraces have no imperfections on the surface and are not standardized. The study highlights a trend by consumers to attribute a value to the feature "local variety", but, obviously, in a real market this value is connected to the specific and intrinsic characteristics possess by a determined landrace. Therefore, the effectiveness of the valorization of local varieties will be better as much as will be high the degree of appreciation that each of them will meet on the market. The feedback received, however, demonstrates the increasing attention of the consumers for high quality and sustainable food, that they can find in the local varieties, products adequate to meet their demand and satisfy their needs. The data presented in this work also underline the potentiality of local varieties for the sustainability of rural communities offering to farmers the opportunity to have access to niche markets and, thus, differentiate the production without losing the incomes. In facts, consumers could be willing to pay a price sufficient to compensate the costs that farmers might incur for the cultivation of landraces. At the same time, the farmers would contribute to maintain on-farm conservation of agricultural biodiversity.

The results also suggest the necessity to involve farmers in marketing training programmes for a better placing of local products in the market. Indeed, in this paper the cultivation of local tomato varieties is proposed as a possible alternative than commercial hybrids for farmers, but it will be of grater interest also to investigate their willingness to participate in a conservation programme for landraces and involve a multidisciplinary group to design strategies taking into account both farmers needs and consumer preferences (Sardaro et al., 2016).

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