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# Characterization of tomato accessions for morphological, agronomic, fruit quality, and virus resistance traits

Stanislava Grozeva, Amol N. Nankar, Daniela Ganeva, Ivanka Tringovska, Gancho Pasev, and Dimitrina Kostova

**Abstract:** Characterization of local germplasm is an effective way to identify elite breeding material and develop improved varieties. This study was aimed to assess 52 tomato accessions comprised of local varieties (28), landraces (8), breeding lines (14), and wild relatives (2), and their characterization for 30 morphological/agronomic, four fruit quality, and tomato mosaic virus (ToMV) resistance traits. Morphological, quality, and ToMV traits were evaluated using phenotyping, biochemical assays, and molecular markers, respectively. Fruit shape and size showed appreciable variation, with fruits varying from rounded to heart shaped and small to big size. Significant variation was observed for fruit weight (1.6–564.8 g), fruits per plant (6.0–174.7), productivity (130.5–5146.5 g), soluble solids (4.1%–8.4%), vitamin C (9.5–46.4 mg·100 g<sup>-1</sup>), antioxidant activity (2.5–9.6 μmol Fe<sup>2+</sup>·g<sup>-1</sup> fresh weight), and total polyphenols (23.9–124.2 GAE·100 g<sup>-1</sup> fresh weight). All accessions were phenotypically screened for the virus resistance in the growth chamber, and CAPS molecular markers were used to identify accessions with ToMV *Tm-2<sup>2</sup>* resistant alleles, and accessions LYC-13, LYC-15, LYC-17, LYC-26, and LYC-52 were identified as resistant. Multivariate analysis of morphological and quality traits showed that 35 principal components contributed to the total variation and the first two and 12 principal components explained 47.2% and 90% of the variation, respectively. The evaluated tomato collection appears to have breeding potential, and around 20% of the accessions in the collection (LYC-6, LYC-17, LYC-18, LYC-26 to LYC-31, and LYC 33) are promising genetic resources for variety development that are enriched with enhanced fruit quality and high yield.

**Key words:** Bulgarian tomato, morphometric diversity (tomato), ToMV, multivariate data visualization, anti-oxidant activity.

**Résumé :** Caractériser le plasma germinal local est une bonne façon d'identifier le matériel génétique supérieur dont on pourrait se servir pour l'hybridation et le développement de meilleures variétés. Les auteurs ont évalué 52 obtentions de tomate incluant des variétés locales (28), des populations naturelles (8), des souches génalogiques (14) et des espèces sauvages apparentées (2), puis les ont caractérisées en fonction de 30 caractères morphologiques ou agronomiques, de quatre caractères liés à la qualité du fruit et de la résistance au virus de la mosaïque de la tomate (ToMV). Les caractères morphologiques et ceux associés à la qualité du fruit ainsi qu'à la résistance au ToMV ont respectivement été évalués par phénotypage, par dosage biochimique et au moyen de marqueurs moléculaires. La forme et la taille du fruit varient passablement, la première allant de sphérique à cordiforme, et la seconde, de petite à grosse. D'importantes variations ont été notées pour le poids du fruit (de 1,6 à 564,8 g), le nombre de fruits par plants (de 6,0 à 174,7), la productivité (de 130,5 à 5 146,5 g), la concentration de solides solubles (de 4,1 à 8,4 %), la teneur en vitamine C (de 9,5 à 46,4 mg par 100 g), le pouvoir antioxydant (de 2,5 à 9,6 μmol de Fe<sup>2+</sup> par g de poids frais) et la concentration totale de polyphénols (de 23,9 à 124,2 équivalents d'acide gallique par 100 g de poids frais). Les auteurs ont examiné le phénotype de toutes les obtentions dans une chambre de croissance afin d'en déterminer la résistance au virus et ont recouru à des marqueurs moléculaires CAPS pour identifier les obtentions portant les allèles *Tm-22* de résistance au ToMV. Les obtentions LYC-13, LYC-15, LYC-17, LYC-26 et LYC-52 résistent au virus. L'analyse multivariable des caractères associés à la morphologie et à la qualité du fruit indique que 35 composantes principales concourent à la variation globale et que les deux premières ainsi que les douze premières composantes dans la liste expliquent respectivement 47,2 % et 90 % de la variation. La collection de tomates évaluée semble présenter un potentiel intéressant pour l'hybridation

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et environ 20 % des obtentions (LYC-6, LYC-17, LYC-18, LYC-26 à LYC-31, LYC 33) sont génétiquement prometteuses en vue de la création de variétés plus productives, aux fruits de meilleure qualité. [Traduit par la Rédaction]

**Mots-clés :** tomate de Bulgarie, diversité morphométrique (tomate), ToMV, visualisation de l'analyse multivariable, pouvoir antioxydant.

## Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important and widely grown vegetable crops. In comparison to other fruits and vegetables, tomatoes are relatively low in antioxidant content, but routine high-level consumption makes them a physiologically relevant source of antioxidants and other chemoprotective compounds (Pérez-Jiménez et al. 2010; Boches et al. 2011). In Bulgaria, local landraces are known for their large fruit size, fleshy texture, traditional taste, and flavor (Ganeva et al. 2014a). Due to constant natural or artificial selection, landraces are well acclimatized and adapted to the local environment but are often not suitable for high input cultivation due to low productivity, poor disease resistance, and lack of uniform fruit quality and morphometric attributes (Fess et al. 2011). Despite these limitations, local landraces are still excellent resources to broaden the genetic variability and for germplasm enhancement.

To enhance any breeding program, a detailed characterization of diverse germplasm is required. Morphological characterization is the first step for the evaluation of genetic diversity and is also important for the conservation and preservation of genetic resources (Terzopoulos and Bebeli 2010; Osei et al. 2014; Figas et al. 2015; Sacco et al. 2015). Breeders and gene bank curators discriminate accessions based on conventional descriptors or traits, which are highly heritable (IPGRI 1996). Conventional descriptors often display a large range of variation, which are useful in classifying accessions into distinct varietal groups and are widely used to describe phenotypic or morphological diversity (Gepts 2006; Upadhyaya et al. 2008). However, agronomic and morphological trait characterization supported by biochemical and molecular analysis has proven useful in varietal identification (Clement et al. 2010; Dias et al. 2013), varietal typification (Pereira-Dias et al. 2020), and assessment of genetic diversity (Khadivi-Khub et al. 2008; Xu et al. 2013; Zhou et al. 2015; Mohan et al. 2016; Lázaro 2018).

Modern elite cultivars are created by rigorous selection and have been very successful in increasing phenotypic diversity as well as increased yield and productivity; however, it has resulted in a noticeable loss of genetic diversity and a significant decline in landraces use (Miller and Tanksley 1990; Williams and St. Clair 1993; Cebolla-Cornejo et al. 2007; Xu et al. 2013; Sacco et al. 2015). Germplasm derived from a narrow genetic base and lack of abundant genetic variation has resulted in inbreeding depression and has caused resistance

breakdown (Poland et al. 2009), deterioration of fruit quality (Salari and Prasad 2010; Glogovac et al. 2012), decreased tolerance to abiotic stresses (Keneni et al. 2012), and subsequent reduced genetic gain. Uses of wild relatives and local landraces have proven effective in finding novel gene sources for important traits and in broadening genetic variation (Rick and Chetelat 1995; Sacco et al. 2015). However, often the utilization of wild species is considered very difficult and time-consuming. Hence, characterization of locally adapted and acclimatized germplasm is one of the effective ways to find promising gene sources and utilize them for the creation of improved varieties.

Although landraces are useful genetic resources for crop improvement (Hawtin et al. 1996; Hoisington et al. 1999; Corrado et al. 2014), the lack of detailed information about their phenotypic characterization, topographical distribution, and genetic relationship to related landraces limit their proper use in breeding programs. The aim of the current work is to characterize a tomato collection, comprised 52 diverse accessions using agronomic, morphological, fruit quality, and virus resistance traits. Our specific objectives were (a) to characterize these accessions for morphological, agronomic, fruit quality, and virus resistance traits; (b) identify accessions those have a high yield, enhanced fruit quality, and resistance to ToMV. Selected accessions with unique and valuable traits could be used in a subsequent breeding program for the development of tomato varieties with improved fruit quality and high yield.

## Materials and Methods

### Germplasm collection

About 52 accessions varying in fruit shape, size, and colour were chosen from the Maritsa Vegetable Crops Research Institute (MVCRI) tomato collection. The selected accessions consisted of local varieties (28), landraces (8), breeding lines (14), and wild relatives (2) as shown in Table 1.

### Experimental design

Field experiments were carried out at the MVCRI, Plovdiv, Bulgaria (N42°10'29", E24°45'42") during 2016 and 2017. Tomato seedlings were transplanted at the beginning of May in a two-row planting scheme with 25–30, 50, and 110 cm plant to plant, row to row, and between row distance, respectively, for determinate genotypes, whereas 30 and 80 cm plant to plant and between row distance, respectively, in one-row planting for indeterminate genotypes. All accessions were grown by following standard production practices described

**Table 1.** List of 52 tomato accessions and population type.

Accession ID	Accession name	Country of origin	Population type	Plant growth habit	Yield per plant	ToMV resistance
LYC-1	Ideal	Bulgaria	Local variety	Indeterminate	Medium	Susceptible
LYC-2	BG Fantazia	Bulgaria	Local variety	Indeterminate	Medium	
LYC-3	Aleno sartse	Bulgaria	Local variety	Indeterminate	Low	Susceptible
LYC-4	1422	Bulgaria	Landrace	Indeterminate	Medium	Susceptible
LYC-5	1300	Bulgaria	Landrace	Indeterminate	Medium	Susceptible
LYC-6	1341	Bulgaria	Landrace	Indeterminate	Medium	Susceptible
LYC-7	874	Bulgaria	Landrace	Indeterminate	Medium	Susceptible
LYC-8	2065	Bulgaria	Landrace	Indeterminate	Low	Susceptible
LYC-9	2066	Bulgaria	Landrace	Indeterminate	Medium	Susceptible
LYC-10	2069	Bulgaria	Landrace	Indeterminate	Medium	Susceptible
LYC-11	24/13	Bulgaria	Local variety	Indeterminate	Medium	Susceptible
LYC-12	24/a	Bulgaria	Local variety	Indeterminate	Medium	Susceptible
LYC-13	937	Bulgaria	Breeding line	Indeterminate	High	Resistant
LYC-14	631	Bulgaria	Breeding line	Semi-determinate	High	Susceptible
LYC-15	Pautalia	Bulgaria	Local variety	Semi-determinate	High	Resistant
LYC-16	799	Bulgaria	Local variety	Indeterminate	Low	Susceptible
LYC-17	800	Bulgaria	Landrace	Indeterminate	Medium	Resistant
LYC-18	605	Bulgaria	Breeding line	Indeterminate	Medium	Susceptible
LYC-19	Pl. karotina	Bulgaria	Local variety	Indeterminate	Medium	Susceptible
LYC-20	Rozovo sartse	Bulgaria	Local variety	Indeterminate	Medium	Susceptible
LYC-21	Rozov blian	Bulgaria	Local variety	Indeterminate	Medium	Susceptible
LYC-22	1090	Bulgaria	Breeding line	Indeterminate	Medium	Susceptible
LYC-23	900	Bulgaria	Breeding line	Indeterminate	High	Susceptible
LYC-24	Izk Alia	Bulgaria	Local variety	Indeterminate	Medium	Susceptible
LYC-25	1923	Bulgaria	Local variety	Indeterminate	High	Susceptible
LYC-26	894750235	USA	Wild	Semi-determinate	Low	Resistant
LYC-27	Elitsa	Bulgaria	Breeding line	Indeterminate	Low	Susceptible
LYC-28	1621-2	Bulgaria	Breeding line	Determinate	High	Susceptible
LYC-29	1620	Bulgaria	Breeding line	Determinate	Medium	Susceptible
LYC-30	1619-2	Bulgaria	Breeding line	Determinate	Medium	Susceptible
LYC-31	83602029	Bulgaria	Breeding line	Indeterminate	Low	Susceptible
LYC-32	2061	Bulgaria	Breeding line	Indeterminate	Medium	Susceptible
LYC-33	894970110	USA	Wild	Semi-determinate	Low	Susceptible
LYC-34	462	Bulgaria	Breeding line	Determinate	Medium	Susceptible
LYC-35	Karobeta	Bulgaria	Local variety	Determinate	Medium	Susceptible
LYC-36	Merkuriy	Bulgaria	Local variety	Determinate	Medium	Susceptible
LYC-37	Spektar	Bulgaria	Local variety	Determinate	Medium	Susceptible
LYC-38	Bononia	Bulgaria	Local variety	Determinate	Medium	Susceptible
LYC-39	Trapezitsa	Bulgaria	Local variety	Determinate	Medium	Susceptible
LYC-40	Solaris	Bulgaria	Local variety	Determinate	Medium	Susceptible
LYC-41	Yana	Bulgaria	Local variety	Determinate	Medium	Susceptible
LYC-42	Milyana	Bulgaria	Local variety	Determinate	Medium	Susceptible
LYC-43	Stela	Bulgaria	Local variety	Determinate	Medium	Susceptible
LYC-44	Topaz	Bulgaria	Local variety	Determinate	Medium	Susceptible
LYC-45	Marti	Bulgaria	Local variety	Determinate	Medium	Susceptible
LYC-46	Venera	Bulgaria	Local variety	Determinate	Medium	Susceptible
LYC-47	Kapri	Bulgaria	Local variety	Determinate	Medium	Susceptible
LYC-48	Bela	Bulgaria	Local variety	Determinate	Medium	Susceptible
LYC-49	Zhaklin	Bulgaria	Local variety	Determinate	Medium	Susceptible
LYC-50	Neven	Bulgaria	Local variety	Determinate	Medium	Susceptible
LYC-51	927	Bulgaria	Breeding line	Determinate	Low	Susceptible
LYC-52	398	Bulgaria	Breeding line	Determinate	Low	Resistant

**Note:** All accessions belong to domesticated species (*Solanum lycopersicum*) except LYC-26 and LYC-33, which are a wild relative of *Solanum peruvianum*.

by [Ganeva et al. \(2014b\)](#). Fertilization, irrigation, and microclimate were the same for all genotypes. The experiment was conducted in a randomized complete block design (RCBD) with three replications. Every accession was represented by nine plants in each replicate.

#### Trait characterization:

All accessions were evaluated by 35 traits including 30 IPGRI morphological and agronomic traits, four fruit quality traits, and ToMV disease resistance screening.

#### Morphological and agronomic traits

Accessions were characterized by 30 morphological and agronomic (fruit weight, number of fruits per plant, and productivity per plant) traits according to tomato descriptors ([IRGRI 1996](#)).

#### Fruit quality

The fruits of indeterminate accessions were harvested multiple times from 1 to 6 trusses, but the samples for fruit quality analysis were only collected from 3 and 4 trusses only. Fruits were collected at full maturity stage in August and were characterized by the following quality traits: Brix or total soluble solids (TSS), and ascorbic acid or vitamin C (Vit C) content in fresh juice, whereas total polyphenols (TP) and antioxidant activity (AA) were estimated in lyophilized material. Total soluble solids were determined by a hand-held refractometer (OPTi Duo, Bellingham Stanley, UK); dry matter (DM) was determined by oven-drying to the constant weight and Vit C was determined by Tillman's reaction ([Tillmans et al. 1932](#)). The extraction procedures for TP and AA were performed according to [Atanasova et al. \(2014\)](#) optimized method. The TP and AA were quantified and measured according to [Singleton and Rossi \(1965\)](#) and [Benzie and Strain \(1996\)](#) methods, respectively.

#### Virus screening

All accessions were screened for resistance against ToMV using the ToMVj strain, which belongs to pathotype P2. Approximately, 10 plants of each accession were grown in trays with peat-perlite mixture in a growth chamber at 22 °C –25 °C and 14/10 h day/night photoperiod. Two independent experiments were carried out with one block layout. These plants were inoculated at the stage of the first true leaf. The viral homogenate was prepared by grinding symptomatic tomato leaves in the buffer (10 g·L<sup>-1</sup> K<sub>2</sub>HPO<sub>4</sub> and 1 g·L<sup>-1</sup> Na<sub>2</sub>SO<sub>3</sub> in dH<sub>2</sub>O, pH 9) in 1:10 w/v ratio. Symptoms were scored each week for a period of 3 wk after inoculation. Discrimination between susceptible and resistant genotypes was done based on a scale: 0—no symptoms, 1—slight mosaic, 2—clear mosaic, 3—heavy mosaic and blisters, and 4—stunted or dead. Back-inoculation

procedures for identifying the systemic spread of the virus were performed on a local host *Nicotiana glutinosa* or *Nicotiana tabacum* 'Xanthi NN'. Accessions with rating 0 and no systemic spread of the virus after back inoculation were considered resistant, and accessions rated with 1, 2, 3, and 4 were virus susceptible. Tomato cultivar 'Ideal' (LYC-1) was used as a susceptible control. In addition to a phenotypic screening of tomato accessions, molecular markers were also used to confirm the results from the phenotypic evaluation. Total genomic DNA was extracted from the upper young leaves of tomato seedlings using a sbeadex kit (LGC genomics). Genotyping was carried out by CAPS marker Tm2RS (5'-TGGAGGGGAATATTTGTGGA-3' and 5'-ACTTCAGACAACCCATTCGG-3') according to [Shi et al. \(2011\)](#). Polymerase chain reaction (PCR) amplifications were conducted in Biorad T100 (Biorad Laboratories) thermocycler in a final volume of 25 µL. Reaction mixture consisted of 1× reaction buffer, 1.5 mmol·L<sup>-1</sup> MgCl<sub>2</sub>, 0.3 mmol·L<sup>-1</sup> dNTPs mix, 0.4 µmol·L<sup>-1</sup> of each primer, 1 U BIOTAQ™ (Bioline Reagents Ltd., London, UK), and 50 ng genomic DNA as a template. PCR thermal cycling was followed as described by [Shi et al. \(2011\)](#), and endonuclease treatment (BoxI, KspAI and Alw21I; Fast Digest, Thermo Fisher Scientific) and electrophoresis of the PCR product were performed according to [Pasev et al. \(2016\)](#). Briefly, discrimination between the susceptible and resistant genotype was determined on the basis of the obtained restriction profile (Supplementary Table S1<sup>1</sup>).

#### Statistical analysis

Analysis of variance (ANOVA) was performed using Proc MIXED, and significant differences were verified with the Duncan test using SAS version 9.2 ([SAS Institute Inc. 2002](#)). A total of 34 traits including morphological, agronomic, and fruit quality traits were used to establish distinct clusters using Ward's coefficient of agglomerative hierarchical clustering in the R program using *dendextend* ([Galili 2015](#)) and circular implementation of dendrogram was done using the *circlize* R package ([Gu et al. 2014](#)). Multivariate analysis of principal component analysis (PCA) parameters was estimated using different R packages including *ggplot2*, *FactoMineR*, *Factoextra*, and *missMDA*.

## Results

### Characterization of tomato accessions by morphological and agronomic traits

The passport data of studied tomato collection provided the basic information of the accessions and described the original morphological traits observed when the accessions were originally collected ([Table 1](#)). Fifty accessions belong to *S. lycopersicum* L. and two accessions (LYC-26 and LYC-33) belong to *Solanum peruvianum* L.

<sup>1</sup>Supplementary data are available with the article at <https://doi.org/10.1139/cjps-2020-0030>.



(wild species). Growth habits of the evaluated accessions were mostly indeterminate (50.0%) and determinate (42.3%), with only 7.7% semi-determinate. Fruit shape wise, the majority of accessions were flattened to rounded (63.5%) and high rounded (26.9%); however, a few accessions were heart shaped (LYC-3, LYC-8, and LYC-20), pear shaped (LYC-32), and roma type (LYC-52). The most common exterior colour of mature fruit was observed to be red (65.4%), followed by pink (11.5%), orange (7.8%), yellow (5.8%), brown (3.8%), green (3.8%), and orange-red (1.9%). Based on fruit size, accessions ranging from very large, large, medium, small, and very small had a share of 19.3%, 25.0%, 28.8%, 15.4%, and 11.5%, respectively. Measurements of productivity per plant revealed that most accessions were moderately yielding (71.2%) or low yielding (19.2%), with only 9.6% of accessions reported as high yielding. A detailed breakdown of morphological, productivity, and fruit quality traits for all evaluated accessions is shown in Supplementary Tables S2, S3, and S4<sup>1</sup>, respectively.

In addition to explaining the morphological and fruit quality trait variation among accessions regardless of their population type, the variation found within local varieties, landraces, breeding lines, and wild relatives were also studied. Local varieties were mostly determinate with rounded red fruits, whereas landraces were indeterminate and of highly flattened to flattened fruit shape (Supplementary Table S2<sup>1</sup>). Breeding lines were composed of determinate and indeterminate growth habit with rounded and high rounded fruits of red, orange, and brown colour (Supplementary Table S2<sup>1</sup>). For productivity traits, local varieties and landraces were more productive than other population types (Supplementary Table S5<sup>1</sup>), and variation for fruit weight (11.1–501.2 g), fruits per plant (8.3–98.2), and productivity (672.2–5146.5 g) was higher within local varieties (Supplementary Table S5<sup>1</sup>). Fruit quality was better in wild species (TSS, 7.9%; AA, 8.9  $\mu\text{mol Fe}^{2+} \cdot \text{g}^{-1}$  FW; and TP, 82.2 mg GAE/100 g<sup>-1</sup> FW) except for Vit C (30.0 mg/100 g<sup>-1</sup>), which was seen higher in breeding lines. However, variation observed for TSS (4.5%–8.2%), Vit C (18.4–46.4 mg/100 g<sup>-1</sup>), AA (3.2–11.8  $\mu\text{mol Fe}^{2+} \cdot \text{g}^{-1}$  FW), and TP (29.2–124.2 mg GAE/100 g<sup>-1</sup> FW) was higher within breeding lines in comparison to local varieties, landraces, and wild species (Supplementary Table S6<sup>1</sup>).

### Hierarchical cluster analysis (HCA)

Cluster analysis of all morphological and agronomic traits identified eight distinct clusters (Fig. 1) regardless of their population type. Among all clusters, only clusters 1, 5, and 7 were distinctly grouped with accessions mainly populated with local varieties and landraces, wild type, and breeding lines, respectively. Clusters 2, 3, 4, 6, and 8 were populated with a mixture of local varieties and breeding lines (Figs. 1 and 2); however, cluster 4 was composed of local varieties except for four breeding lines (LYC-14, LYC-18, LYC-51, and LYC-52). Cluster 1

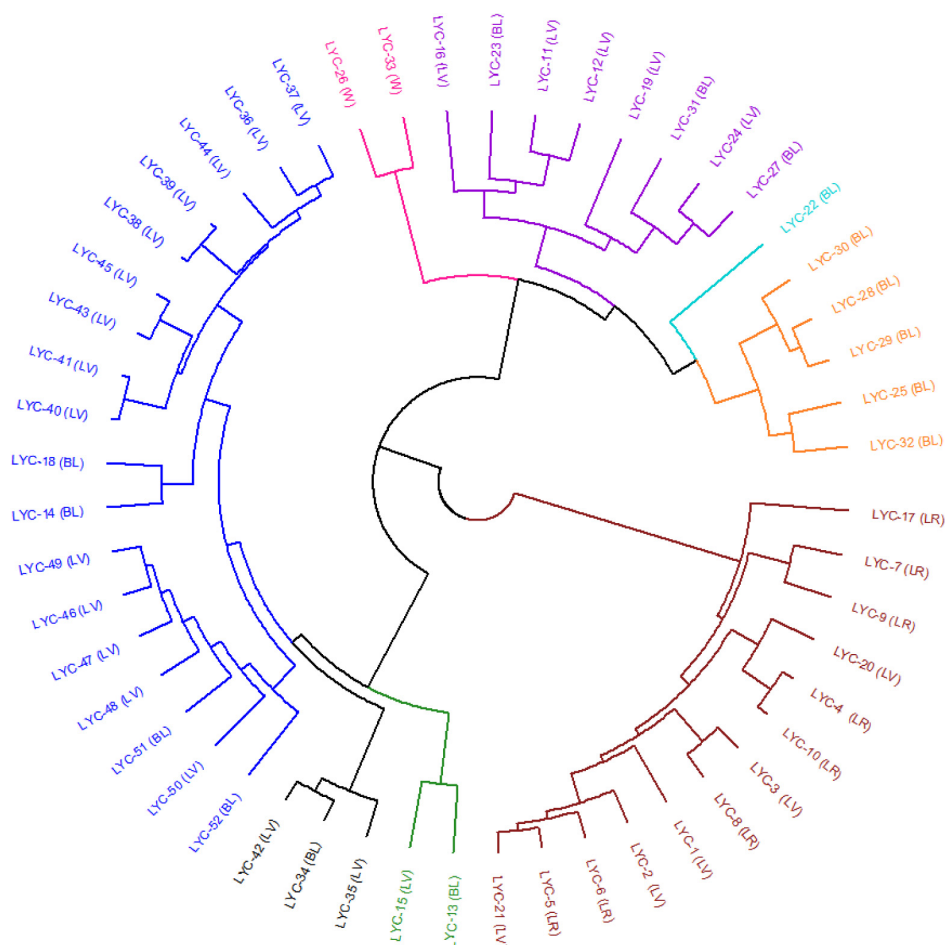
consisted of 13 accessions, which are typical landraces, with indeterminate growth habit, and large to very large fruits (Fig. 2). Cluster 2 included two accessions (LYC-13 and LYC-15) distinguished by semi-determinate growth habit and high rounded fruits, and cluster 3 with round-fruited accessions (LYC-34, LYC-35, and LYC-42). Cluster 4 covered 21 accessions with determinate growth habit and medium to large fruits. Cluster 5 was distinct in nature with the inclusion of two wild relatives (LYC-26 and LYC-33) belonging to *S. peruvianum*. Cluster 6 was comprised of 10 accessions consisting of small-fruited “cherry type”, whereas accession LYC-22 distanced from other accessions and formed a cluster 7 separately. Cluster 8 was an admixture of accessions with mostly small fruits and variable round, oblong, and pear shapes (Fig. 2). Cluster-wise accession comparisons for productivity and fruit quality traits are shown in Supplementary Tables S3 and S4<sup>1</sup>, respectively.

### Variation in productivity, productivity components, and fruit quality

Once distinct clusters were identified, each cluster was assessed for variation in productivity and yield components (fruit weight and number of fruits per plant) for 2016 and 2017 (Table 2), whereas fruit quality was only evaluated during 2016 (Table 3). The main effect of accession was tested across clusters 1–6 and 8, but cluster 7 was not included since it was represented by a single accession. Between clusters, accessions were significantly different for fruit weight and productivity across years, whereas the interaction between accession and year ( $A \times Y$ ) was also much different for all yield components except for fruit weight (Table 2). Cluster wise, accessions in all clusters showed significant differences in respective years except for clusters 2 and 5. The  $A \times Y$  interaction for fruit weight was significant in clusters 5 and 6, whereas for fruits per plant, the  $A \times Y$  interaction was significant in clusters 1, 4, and 6, and for productivity, only cluster 5 showed significant  $A \times Y$  interaction (Table 2). In regard to fruit quality, accessions populated in clusters 1, 4, 6, and 8 discerned significant differences for TSS, Vit C, FRAP, and TP except for nonsignificant differences for FRAP in cluster 8 (Table 3). Accessions in clusters 2, 3, and 5 showed nonsignificant differences for all fruit quality traits except for Vit C in clusters 3 and 5 (Table 3).

Across clusters, a wide range of variation was seen for productivity traits (Supplementary Table S3<sup>1</sup>) where fruit weight, fruits per plant, and productivity varied from 1.6 (LYC-26) to 564.8 g (LYC-5), 6 (LYC-3) to 147.2 (LYC-30), and 130.5 (LYC-26) to 5146.5 g (LYC-37), respectively. Irrespective of any cluster, accessions with heart-shaped tomatoes had largest and bigger fruits (LYC-2, LYC-4, LYC-5, LYC-9, and LYC-20), while wild species (LYC-26 and LYC-33) had the smallest fruits (Supplementary Table S2<sup>1</sup>). Accessions with small rounded fruits (LYC-28, LYC-29, and LYC-30) had the highest number of fruits per

**Fig. 1.** Relatedness dendrogram based on 30 agronomic and morphological traits, four fruit quality traits, and ToMV resistance. Population type of each accession is accompanied with the accession name and population types are abbreviated as BL for breeding lines, LV for local varieties, LR for landraces, and W for wild species. [Colour online.]



plant, whereas heart-shaped large-fruited accessions had the least number of fruits per plant (Supplementary Table S3<sup>1</sup>). Small rounded (LYC-36, LYC-37, and LYC-38), large flattened (LYC-9), and large heart-shaped (LYC-20) accessions showed the highest productivity, while wild species had low productivity (Supplementary Table S3<sup>1</sup>).

In regard to fruit quality, very small and round fruit shape accessions (LYC-27, LYC-28, LYC-29, LYC-30, and LYC-31) and wild species (LYC-26 and LYC-33) tended to have a high content of TSS, Vit C, AA, and TP (Supplementary Table S4<sup>1</sup>). Regardless of clusters, TSS, Vit C, AA, and TP ranged from 4.1% (LYC-49) to 8.4% (LYC-26), 9.5 (LYC-26) to 46.4 mg·100 g<sup>-1</sup> (LYC-31), 2.5 (LYC-4) to 11.8 µmol Fe<sup>2+</sup>·g<sup>-1</sup> FW (LYC-27), and 23.9 (LYC-4) to 124.2 mg GAE·100 g<sup>-1</sup> FW (LYC-27), respectively. Trait wise, very small and round-shape accessions (LYC-26, LYC-27, LYC-28, LYC-29, and LYC-30) tended to have a higher amount of TSS, while slightly flattened (LYC-5) and medium-high rounded accessions (LYC-49) had the least TSS (Supplementary Table S4<sup>1</sup>). The highest Vit C content was reported in LYC-21, LYC-19, LYC-24, LYC-27, and LYC-7, and the lowest was reported in

LYC-26 and LYC-33 (wild species). The highest AA and TP content was seen in LYC-27, LYC-29, and LYC-31, while the lowest was in LYC-4, LYC-7, and LYC-2 (Supplementary Table S4<sup>1</sup>). AA and TP are highly correlated, as the accessions with high AA tended to have high TP too.

#### Resistance to ToMV within a collection of 52 tomato accessions

Biological screening for resistance to ToMV revealed that LYC-13 and LYC-52 (breeding lines), LYC-15 and LYC-17 (local varieties), and LYC-26 (wild species) were asymptomatic without a systemic spread of the virus after back-inoculation in the phenotypic evaluation (Supplementary Table S3<sup>1</sup>). These accessions were considered resistant, while other accessions with typical systemic symptoms (rating 1–4) were referred to as susceptible. Genotyping of the *Tm-2* locus by CAPS marker revealed the *Tm-2*<sup>2</sup> allele in the resistant accessions (Supplementary Table S3<sup>1</sup>). The primary PCR fragment of 703 for these genotypes were digested into 458 and 245 bp fragments with the KspAI enzyme, while it remained uncut when treated with BoxI and Alw211

**Fig. 2.** Diversity of fruit shape, size, and colour within each distinct cluster. Population type of each accession is accompanied with the accession name and population types are abbreviated as BL for breeding lines, LV for local varieties, LR for landraces, and W for wild species. [Colour online.]



(Supplementary Table S1<sup>1</sup>), suggesting that the *Tm-2*<sup>2</sup> allele is in the homozygous state (Fig. 3). The remaining 47 genotypes possessed the susceptible *tm-2* allele in a homozygous condition rendering a pattern consisting of two fragments when cut with BoxI – 538 and 165 bp.

The resistant accessions were scattered in four different clusters due to their differences in morphological and agronomic traits. LYC-13 and LYC-15 were grouped in cluster 2, with indeterminate growth habit and high round-shape fruits; LYC-17 from cluster 1 had round-shape fruits, while LYC-26 from cluster 5 was a wild accession and LYC-52 from cluster 4 were roma type.

#### Principal component analysis

The PCA with combined morphological, fruit quality, and virus resistance traits identified a total of 35 principle components that contributed to the total variation. Accession by cluster ( $A \times C$ ) ellipse biplot allowed us to understand if the accessions belonging to each cluster can be separated (Fig. 4). Clusters 2 (LYC-13 and LYC-15), 5 (LYC-26 and LYC-33), and 7 (LYC-22) could not calculate any ellipse since there were not enough points to estimate the same; however, clusters 1 and 4 were distinctly separated, whereas clusters 6 and 8 were overlapped together. Accessions populated in cluster 1

(LYC-1-10, LYC-17, LYC-20, and LYC-21) were big fruited and were mainly separated by large plant size, while accessions in cluster 4 (LYC-14, LYC-18, LYC-36-LYC-41, and LYC-43-LYC-52) were distinctly separated by red coloured fruits, medium fruit yield, and round to high round fruit shape. Accessions from cluster 3 (LYC-34, LYC-35, and LYC-52) were also partly overlapped with cluster 4, and ToMV resistance seems to be correlated with medium fruit yield, whereas accessions from clusters 6 and 8 were mainly characterized by fruit quality traits (Fig. 4). The first two components explained 47.2% of the variation (Fig. 4 and Table 4) with respective components explaining 31.3% and 15.9% variation. The PC1 positively correlated with plant and fruit morphological traits except for style position, fruit shape, and ripened fruit colour, varietal type, fruits per plant, while all fruit quality traits were negatively correlated (Table 4). The PC2 variance was explained by plant growth habit, plant size, leaf attribute, inflorescence type, presence of green shoulder, and fruit quality traits of TSS, Vit C, antioxidant activity, and total phenols (Table 4). Traits contributing to PC2 were positively correlated except leaf type, fruit shape, size, blossom end shape, fruit set, flowering, maturity earliness, and productivity (Fig. 4 and Table 4).



**Table 2.** Analysis of variance (ANOVA) of fruit weight, plant productivity, and fruits per plants for identified clusters and across accessions during 2016, 2017, and across years.

Cluster	Effect	DF			Fruit Weight (gm)			Fruits per Plants			Productivity		
		2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled
Across Clusters	Accession	51	51	51	84.0***	131.2***	456.2***	36.7***	214.6***	1.19	35.9***	2.99***	3.93***
	Rep	2	2	2	1.27	0.6	0.98	2.39	3.49*	3.23*	0.16	0.62*	0.66
	Year	—	—	1	—	—	1.78	—	—	1.96	—	—	0.64
	A × Y Inter	—	—	51	—	—	0.45	—	—	724.9***	—	—	1.79**
Cluster 1	Accession	12	12	12	12.5***	17.7***	71.9***	15.5**	29.7***	20.1***	3.48**	1.95	3.31**
	Rep	2	2	2	1.20	1.16	1.23	0.13	0.96	0.09	0.56	1.30	0.18
	Year	—	—	1	—	—	0.85	—	—	4.24	—	—	3.62
	A × Y Inter	—	—	12	—	—	0.39	—	—	1.94*	—	—	1.18
Cluster 2	Accession	1	1	1	0.93	0.28	0.05	5.32	0.44	3.74	52.9*	0.32	3.93
	Rep	2	2	2	2.23	1.99	1.49	2.41	0.25	2.01	0.33	0.33	0.39
	Year	—	—	1	—	—	12.4***	—	—	9.82	—	—	0.02
	A × Y Inter	—	—	1	—	—	0.78	—	—	1.25	—	—	1.62
Cluster 3	Accession	2	2	2	11.4***	30.1***	32.3*	1.00	5.44	0.54	5.71	663.3***	23.4*
	Rep	2	2	2	0.97	1.12	0.45	0.43	1.0	1.50	0.37	42.0*	1.95
	Year	—	—	1	—	—	1.52	—	—	0.70	—	—	6.94
	A × Y Inter	—	—	2	—	—	0.86	—	—	3.79	—	—	1.25
Cluster 4	Accession	17	17	17	35.8***	41.1***	178.5***	22.9***	21.6***	1.06	11.7***	1.09	1.05
	Rep	2	2	2	0.35	1.46	1.13	0.78	0.59	1.37	2.43	0.64	0.70
	Year	—	—	1	—	—	3.69	—	—	1.51	—	—	0.78
	A × Y Inter	—	—	17	—	—	0.43	—	—	12 906***	—	—	1.23
Cluster 5	Accession	1	1	1	0.69	4.67	0.30	3.77	0.69	20.25	5.49	4.11	0.24
	Rep	2	2	2	0.59	0.86	0.62	3.77	0.86	2.61	0.21	0.52	0.80
	Year	—	—	1	—	—	3.16	—	—	2.25	—	—	2.24
	A × Y Inter	—	—	1	—	—	5.06*	—	—	0.12	—	—	8.60*
Cluster 6	Accession	7	7	7	168.3***	424.3***	33.7***	42.6***	36.0***	5.96*	26.4***	28.1***	56.0***
	Rep	2	2	2	0.83	0.98	1.01	0.43	1.08	1.57	0.26	0.52	0.36
	Year	—	—	1	—	—	0.34	—	—	0.01	—	—	0.10
	A × Y Inter	—	—	7	—	—	15.0***	—	—	11.7***	—	—	0.99
Cluster 8	Accession	4	4	4	97.2***	120.1***	279.1***	13.6***	103.2***	127.7***	6.29**	5.45*	17.8**
	Rep	2	2	2	1.31	0.81	0.73	3.92	1.73	3.50*	2.54	1.03	1.09
	Year	—	—	1	—	—	2.20	—	—	2.57	—	—	0.02
	A × Y Inter	—	—	4	—	—	0.74	—	—	0.33	—	—	0.54

**Note:** Asterisks \*, \*\*, and \*\*\* indicate significance at  $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.001$ , respectively. ANOVA for cluster 7 was not analyzed as it had only a single accession.

## Discussion

Knowledge about germplasm diversity is critical for germplasm conservation, utilization, and varietal development. The primary goal of any variety development and crop improvement program is to target yield and yield components, but breeding for early maturity, fruit quality, and multiple stresses is equally important. To achieve this balance, any breeding program needs to have a strong pre-breeding genepool where sufficient genetic recombinants are being created for future selection. Creating a strong pre-breeding genepool requires the detailed characterization of the germplasm that has potential traits of breeding interest. Conventionally, morphological and agronomic traits have been used in phenotypic evaluation (Frankel 1984), and the same sets of traits were also found as

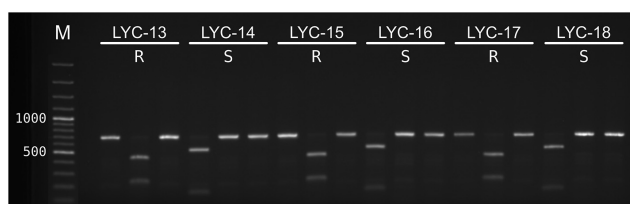
suitable for detailed accession characterization in this study.

The results obtained from this study show a rich diversity across the evaluated tomato collection, and descriptors displayed large variations in fruit shape, size, productivity, yield components, and fruit quality. These results suggest that the presented collection has considerable agro-morphological variation, which is in concurrence with earlier studies that used agronomic and morphological traits (Mavromatis et al. 2013; Omar et al. 2019; Salim et al. 2020), fruit morphology (Nankar et al. 2020), and fruit quality (Mavromatis et al. 2013; Sumalan et al. 2020) to characterize the tomato collections. Also, variability reported for morphometric traits of fruit shape, size, and colour indicates that tomato producers prefer fruits of peculiar fruit types, and this

**Table 3.** Analysis of variance (ANOVA) of fruit quality of TSS, Vit C, AA, and TP for identified clusters and across accessions during 2016.

Cluster	Effect	DF	TSS	Vit C	AA	TP
Across clusters	Accession	51	73.8***	31.5***	44.7***	68.9***
	Rep	2	0.32	20.7***	8.82**	25.1***
Cluster 1	Accession	12	10.5***	13.5***	17.6***	116.3***
	Rep	2	0.17	4.41	2.71	51.9***
Cluster 2	Accession	1	9.0	0.08	4.07	13.8
	Rep	2	9.0	3.72	3.79	126.3*
Cluster 3	Accession	2	5.29	34.1*	0.52	8.68
	Rep	2	0.57	55.2*	0.03	29.7*
Cluster 4	Accession	17	17.15***	9.97***	5.76***	7.79***
	Rep	2	0.46	4.96*	5.31*	9.59**
Cluster 5	Accession	1	Infy***	906.7***	53.9	1.81
	Rep	2	Infy***	2272.1**	36.9	0.48
Cluster 6	Accession	7	150.3***	13.2**	46.9***	43.7***
	Rep	2	1.48	8.05*	0.71	3.42
Cluster 8	Accession	4	53.1***	181.2***	2.14	41.8**
	Rep	2	1.64	22.4**	0.62	0.01

**Note:** Asterisks \*, \*\*, and \*\*\* indicate significance at  $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.001$ , respectively. ANOVA for cluster 7 was not analyzed as it had only a single accession.

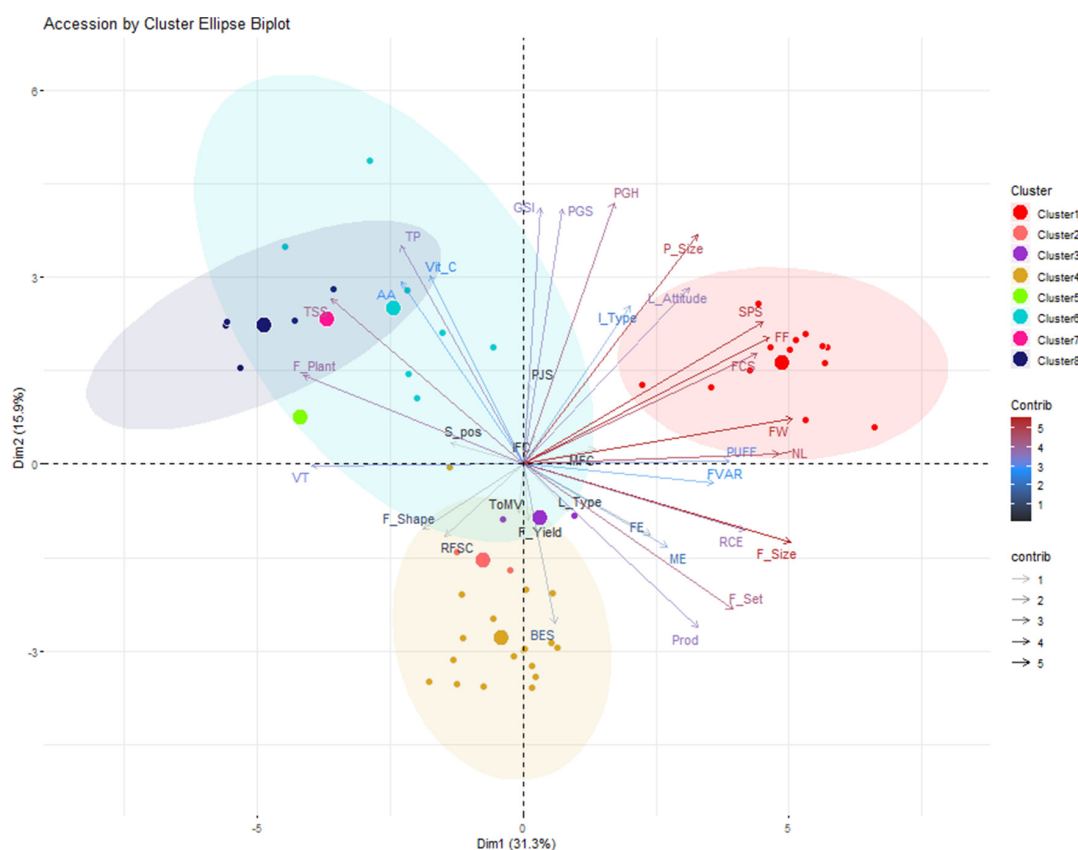
**Fig. 3.** CAPS marker for identification of alleles in *Tm-2* locus. A PCR fragment of 703 bp from six tomato accessions (designated as LYC-13 to LYC-18) digested with three restriction enzymes (Box I, KspAI, and Alw21I) in separate reactions. S, susceptible (*tm-2/tm-2*); R, resistant (*Tm-2<sup>2</sup>/Tm-2<sup>2</sup>*) genotypes. M, GeneRuler™ 100 bp Plus DNA Ladder (Thermo Scientific Inc.).

information could be used as a base for the development of varieties that has desirable features for the targeted Balkan market segment (Nankar et al. 2020; Sumalan et al. 2020). The traits with low variation within the collection are leaf type and style position, which are typical for *S. lycopersicum*. Similar results with a large variation for some fruit characteristics were also reported in previous tomato studies, and these findings also corroborate with our findings (Mazzucato et al. 2010; Cortés-Olmos et al. 2014).

Cluster analysis based on 30 morphological and agronomic traits divided the studied accessions into eight distinct groups. Cluster analysis identified accessions those populated in distinct clusters based on their shared similarity and genetic relatedness (Mercati et al. 2014; Figàs et al. 2015; Nankar et al. 2020). This would

likely allow us to use these cluster-specific accessions to breed for specific traits of interest such as accessions LYC-28, LYC-29, and LYC-30, which has enhanced fruit quality and a large number of fruits could be useful to breed for fruit quality. Accessions from cluster 1 (LYC-1–LYC-10, LYC-17, LYC-20, and LYC-21) have peculiar fruit taste, texture or fleshiness, fruit shape, and size, which is pertinent to Bulgaria, and this would allow us to breed or improve further by developing potential hybrids between these accessions. Accessions belonging to landraces, small-fruited types, and wild species were well distinguished. Accessions LYC-16, LYC-19, and LYC-22 formed three single clusters due to fruit attribute variability, while LYC-22 featured by potato leaf. Applied cluster analysis does not distinguish local varieties and breeding lines from other accessions and consolidated them in one group, possibly due to close fruit morphological resemblance. According to population type, clusters 2, 3, 4, 6, and 8 comprised local varieties and breeding lines; however, accessions populated in clusters 4 and 6 were mostly local varieties, while accessions populated in clusters 8 and 1 were breeding lines and landraces, respectively. This suggests that the fruit morphology was the basis for the establishment of different cultivar groups regardless of their population type, and it has been priorly used for cultivar grouping and demonstrated its usefulness in varietal typification across tomato (Díez and Nuez 2008; Gonzalo et al. 2009; Nankar et al. 2020), pepper (Tripodi and Greco 2018; Nankar et al. 2019), and eggplant (Hurtado et al. 2013, 2014). According to fruit morphology, Parisi et al. (2016)

**Fig. 4.** PCA accession by cluster ellipse biplot displaying accessions categorized based on identified clusters; each colour and assigned symbol represents accessions belonging to specific clusters. Accessions belonging to clusters 1, 2, 3, 4, 5, 6, 7, and 8 are shown in red, orange, purple, gold, green, light blue, pink, and dark blue coloured dots, respectively. Traits contributing to PC1 and PC2 are also assigned different gradient colours with a gradient ranging from 1, 3, and 5 as dark gray, blue, and red-brown, respectively. The traits included in PCA are abbreviated as PGH, plant growth habit; P\_Size, plant size; L\_Attitude, leaf attitude; L\_Type, leaf type; I\_Type, inflorescence type; S\_Pos, style position; F\_Shape, fruit shape; IFC, immature fruit colour; PJS, presence of jointless pedicel; MFC, mature fruit colour; PGS, presence of green shoulder; GSI, green shoulder intensity; FF, fruit fasciation; F\_Size, fruit size; SPS, shape of pistil scar; BES, blossom end shape; RCE, ribbing at calix end; RFSC, ripened fruit skin colour; FCS, fruit cross-section; NL, number of locules; PUFF, puffiness; VT, varietal type; FVAR, fruit variation within a plant; F\_Set, fruit set; FE, flowering earliness; ME, maturity earliness; F\_Yield, fruit yield; Prod, productivity; F\_Plant, fruits/plant; FW, fruit weight; ToMV, tomato mosaic virus; Vit C, vitamin C; TSS, total soluble solids; AA, antioxidant activity; TP, total phenols. [Colour online.]



distinguish two ‘Sorrento’ morphotypes, and [Corrado et al. \(2014\)](#) established that landraces had a genetic structure that is mainly related to fruit type.

Tomato fruits contain many compounds that have antioxidant potential such as ascorbic acid, polyphenols, carotenoids, and other secondary metabolites ([Lavelli et al. 2000](#); [Tyssandier et al. 2004](#)). In the current study, analytical results for TSS, Vit C, AA, and TP obtained during 2016 discerned high variability between accessions, even within each cluster, indicating that the accession selection with a high content of bioactive components is possible; however, a one-year evaluation is not enough to study the year effect on fruit quality, and further investigation is needed. A multi-location and multiyear experiment is envisaged to comprehend the genotype by environment ( $G \times E$ ) interaction and to check the accessions stability for fruit quality in different environments and seasons. Fruit quality and size

have been seen to be negatively linked, and apparently accessions with smaller fruit size have better fruit quality (LYC-26, LYC-27, LYC-31, and LYC-33). Our results were similar to previous findings indicating that the fruit size and antioxidants content is negatively linked, and small-fruited tomato genotypes possess a higher amount of biochemical compounds ([Willcox et al. 2003](#); [Slimestad and Verheul 2005](#)). These findings would likely be useful to breeding programs in developing varieties that are enriched with enhanced fruit quality, nutritional components, and desirable fruit size.

Any successful tomato variety or hybrid requires a stable resistance against major viral diseases, and resistance to ToMV has become mandatory in contemporary tomato breeding. Conventionally, breeders have relied on the *Tm-2<sup>2</sup>* allele as a resistance source to three known ToMV pathotypes of P0, P1, and P2 ([Hull 2001](#)). Up to now, *Tm-2<sup>2</sup>* has been successfully employed in the

**Table 4.** PCA descriptor trait contribution (features), correlation coefficient ( $R^2$ ), eigenvector, and eigenvalues for principal components 1, 2, and 3.

Trait	Features			Correlation coefficient ( $R^2$ )			Eigenvector			Eigen value	Variance (%)	Cumulative variance (%)
	PC1	PC2	PC3	PC1	PC2	PC3	1	2	3			
Plant growth habit	0.92	10.80	2.23	0.32	0.78	-0.25	0.09	-0.33	-0.18	10.94	31.25	31.25
Plant size	3.39	8.34	2.43	0.61	0.68	-0.26	0.16	-0.3	-0.19	5.58	15.94	47.19
Leaf attitude	3.06	4.92	0.60	0.58	0.52	-0.13	0.17	-0.23	-0.13	2.89	8.24	55.43
Leaf type	0.22	0.34	0.03	0.16	-0.14	0.03	0.05	0.06	0.01	2.22	6.34	61.77
Inflorescence type	1.28	3.96	7.88	0.37	0.47	-0.48	0.11	-0.21	-0.29	2.07	5.91	67.68
Style position	0.58	0.07	14.55	-0.25	0.06	-0.65	-0.08	-0.03	-0.32	1.83	5.23	72.91
Fruit shape	1.10	0.67	0.05	-0.35	-0.19	0.04	-0.11	0.08	-0.01	1.30	3.71	76.63
Immature fruit exterior	0.02	0.13	5.64	0.04	0.08	0.40	0.01	-0.05	0.25	1.22	3.47	80.10
Jointless pedicel	0.00	1.00	0.33	0.02	0.24	0.10	0.01	-0.09	0.07	1.12	3.20	83.30
Matured fruit exterior	0.59	0.04	2.66	0.25	0.05	0.28	0.1	-0.02	-0.02	0.88	2.53	85.82
Greenback presence	0.17	10.30	6.98	0.14	0.76	0.45	0.04	-0.33	0.24	0.67	1.92	87.74
Intensity of greenback	0.03	10.40	8.34	0.06	0.76	0.49	0.01	-0.33	0.27	0.63	1.79	89.53
Fruit fasciation	6.74	2.56	0.24	0.86	0.38	-0.08	0.26	-0.17	-0.05	0.59	1.67	91.20
Fruit size	7.95	0.96	0.65	0.93	-0.23	0.14	0.28	0.08	0.08	0.48	1.36	92.56
Pistil scar shape	6.37	3.23	0.07	0.83	0.42	-0.05	0.25	-0.19	-0.05	0.43	1.22	93.78
Blossom end shape	0.11	4.03	0.38	0.11	-0.47	-0.10	0.03	0.21	-0.09	0.37	1.07	94.85
Ribbing at calix end	5.48	0.68	0.23	0.77	-0.20	0.08	0.23	0.08	0.07	0.30	0.87	95.71
Ripened fruit colour	0.68	0.82	8.93	-0.27	-0.21	0.51	-0.08	0.07	0.32	0.29	0.82	96.53
Transverse fruit section	6.11	1.93	0.03	0.82	0.33	0.03	0.24	-0.14	0.01	0.22	0.62	97.15
Number of locules	7.27	0.02	1.30	0.89	0.03	0.19	0.26	0	0.14	0.19	0.54	97.69
Puffiness	4.75	0.00	0.32	0.72	0.01	-0.10	0.22	0	-0.06	0.16	0.45	98.14
Varietal type	4.98	0.00	0.04	-0.74	0.00	0.03	-0.22	-0.01	0.05	0.14	0.39	98.53
Fruit variation	4.01	0.05	1.14	0.66	-0.05	0.18	0.2	0.02	0.14	0.11	0.30	98.83
Fruit set	4.89	3.35	1.28	0.73	-0.43	0.19	0.22	0.19	0.14	0.10	0.29	99.12
Flowering earliness	1.80	0.81	4.90	0.44	-0.21	-0.38	0.14	0.09	-0.19	0.08	0.22	99.35
Maturity earliness	2.31	1.10	4.00	0.50	-0.25	-0.34	0.15	0.11	-0.17	0.07	0.19	99.54
Fruit yield	0.00	0.51	1.40	0.02	-0.17	0.20	0.01	0.07	0.09	0.05	0.15	99.70
Productivity	3.40	4.23	2.02	0.61	-0.49	0.24	0.2	0.18	0.12	0.04	0.10	99.80
Fruits per plant	5.35	1.24	0.17	-0.77	0.26	0.07	-0.28	-0.02	-0.05	0.02	0.06	99.86
Fruit weight	8.05	0.32	0.18	0.94	0.13	0.07	0.29	0.05	0.11	0.02	0.05	99.91
ToMV ( $Tm-2^2$ )	0.00	0.38	15.97	-0.02	-0.15	-0.68	-0.01	0.06	-0.42	0.01	0.03	99.94
Vit C	0.96	5.64	3.59	-0.32	0.56	0.32	-0.08	-0.25	0.23	0.01	0.02	99.96
Dry matter	4.10	4.34	0.14	-0.67	0.49	-0.06	-0.19	-0.16	0.01	0.01	0.02	99.98
Anti-oxidant activity	1.65	5.27	1.27	-0.42	0.54	-0.19	-0.12	-0.23	-0.02	0.00	0.01	99.99
Total phenols	1.67	7.54	0.01	-0.43	0.65	-0.02	-0.13	-0.28	0.03	0.00	0.01	100.00

development of ToMV-resistant tomato germplasm (Lee et al. 2015). Discrimination of the homozygosity of the  $Tm-2^2$  allele using molecular markers has proven a reliable approach to accelerate the breeding process. Currently, several markers linked to this locus are available for screening tomato germplasm (Dax et al. 1998; Sobir et al. 2000; Arens et al. 2010; Shi et al. 2011; Patel et al. 2015); however, their efficacy for identifying resistance alleles needs to be tested. Earlier published studies seeking resistance against ToMV have shown that the CAPS markers have high efficacy (Shi et al. 2011; Ullah et al. 2019). Reliability of the CAPS marker in investigated germplasm will reduce the tracking of  $Tm-2^2$  resistance allele during the selection process and speed up the breeding cycle (Pasev et al. 2016).

In the current study, resistant  $Tm-2^2$  allele was found in accessions LYC-13, LYC-15, LYC-17, LYC-26, and LYC-52, and these accessions were of different genetic backgrounds. Resistant accessions of *S. lycopersicum* (LYC-13, LYC-15, LYC-17, and LYC-52) can be used for heterosis and combine with other high yielding varieties. Accessions LYC-13 and LYC-15 are high-yielding accessions with indeterminate and semi-determinate growth habit with vigorous plants and medium rounded pink and red fruits, respectively. LYC-17 is a medium-yielding indeterminate accession that has large plants with very big flattened fruits of pink colour. LYC-26 (wild species) is a low-yielding semi-determinate accession with intermediate plant size and very small round green fruits. Low-yielding LYC-52 is a determinate accession with smaller plants and medium-size red fruits.



A combination of high round (LYC-13 and LYC-15) and round shape (LYC-17) indeterminate roma-type accessions could be a good candidate for salad segment breeding programs. Wild species LYC-26 has enhanced fruit quality and could be very useful for wide hybridization and breeding lines development, which are enriched with enhanced fruit quality (Rick and Chetelat 1995; Chen et al. 1999); however, genetic barriers and desirable crossing patterns may limit the chance of obtaining successful interspecific crosses (Ullah et al. 2019). The low yield and unusual fruit shape of LYC-52 may make it a less prospective breeding line in future breeding.

## Conclusion

The evaluated accessions from this study showed that some accessions possess various valuable characteristics including ToMV resistance, improved agronomic traits, and enhanced fruit quality traits. This germplasm collection comprises useful breeding material that could be used as parental genotypes or as pre-breeding material in future variety development for peculiar fruit shape, size, colour, and flavor desirable for the local niche market.

## Conflict of Interest

The authors declare that they have no conflict of interest.

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## Author Contributions

- Study conception and design were done by S. Grozeva and D. Ganeva.

- Material preparation, data collection, and analysis were performed by S. Grozeva, D. Ganeva, I. Tringovska, G. Pasev, A.N. Nankar, and D. Kostova.
- The first draft of the manuscript was written by S. Grozeva, and all authors commented on previous versions of the manuscript.
- All authors read and approved the final manuscript.

## Data Availability

The datasets generated during and (or) analyzed during the current study are available from the corresponding author on reasonable request.

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