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Genetic diversity and population structure of modern Bulgarian tomato: Insights from molecular and biochemical data

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Abstract

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Genetic variation in eight modern Bulgarian tomato varieties and advanced breeding lines showing morphological and biochemical differences was assessed using 182 simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers in 19 genes involved in diverse metabolic pathways associated with quality and other agronomic traits. The genetic diversity within the collection based on SSRs was relatively low (0.368) but similar to that observed with SNP markers (0.316), with a mean genetic diversity estimated from both marker types of 0.3670. Nei's genetic distance based on both marker types varied from 0.1973 between Plovdivska karotina and L21β to 0.7788 between IZK Alya and L984. The grouping in the cluster analysis and the inferred population structure reflected to some extent the morphological and biochemical characteristics of the studied tomato genotypes. The most diverse genotypes are IZK Alya and lines 1140 and 1116. The observed grouping of Plovdivska karotina and L21β in a common sub-cluster of the UPGMA dendrogram reflects their similar morphological and biochemical composition, especially of β-carotene content, which is an important antioxidant that may prevent some chronic diseases, like various cancers and cardiovascular diseases. The genetic distance information for the studied collection could be useful for implementation of further tomato breeding strategies towards improvement of nutritious and yield related traits as well for human diet, farmers and processing industry in Bulgaria.

Keywords: tomato (*S. lycopersicum* L.); molecular markers*:* SSR – (Simple sequence repeats); SNP – (Single nucleotide polymorphism); genotyping; biochemical parameters; genetic diversity; population structure

Introduction

Tomato, ranking first among vegetable crop species worldwide, accounts for 16% of the global vegetable production, which is over 192 million metric tons annually (Food and Agriculture Organisation [FAO] 2024). The global tomato market is projected for significant growth. Its contribution is estimated at about $190 billion in 2025 in value-added output to the economy with forecasts potentially to exceed $300 billion by the early 2030s (FAO, 2024). It is considered as the richest source in the human diet with dietary fiber, antioxidant vitamins (A, C, E), minerals, lycopene, β-carotene (Frusciante et al., 2007). Therefore, beyond the tomato's economic importance, it possesses anticancer and anti-cardi-

ovascular properties (Bhuvaneswari and Nagini, 2005). The key objectives of tomato breeders improving productivity, stress tolerance, and fruit quality rely on effective utilization the intraspecific genetic diversity (Bauchet and Causse, 2012; Fernie et al., 2006). However, the intensive breeding programs have caused a severe loss of genetic variability (Kulus, 2022). This requires to search for new donors of valuable alleles/traits among tomato genotypes from different collections at both, national and international level that can be used as foundation for improvement of existing germplasm to ensure food security and the adaptivity to changeable environmental conditions.

The progress in molecular biology and development of new generation sequencing technologies in the last 2 decades has created a new era in characterization of the genetic diversity present in both wild and cultivated tomato species. The new molecular tools like simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers became invaluable in providing detailed information about genetic relationships, population dynamics, and complex genetic architectures. This has led to tremendous progress in the mapping of chromosome regions controlling important traits utilizing the genetic diversity of both wild and cultivated species (Bauchet et al., 2017).

Bulgaria has a rich collection of tomato genotypes collected, preserved and developed in the Maritsa vegetable crops research institute (MVCRI), Plovdiv and the Institute of plant genetic resources (IPGR), Sadovo. These genetic resources have been subjected to extensive phenotypic evaluation for important traits, including fruit quality and resistance to biotic and abiotic stress. However, up to now a limited number of molecular studies have been performed using SSR, ISSR and AFLP markers on small number of genotypes, including also F1 hybrids (Todorovska et al., 2014; Ivanova et al., 2014; Tomlekova and Balacheva, 2017; Aziz et al., 2021; Bojinov et al., 2024). The lack of extensive molecular research means that more advanced investigations are needed to fully understand the genetic diversity of these valuable tomato collections and to develop effective breeding strategies.

Therefore, this study aimed at evaluation of genetic diversity and population structure of 8 modern tomato varieties and lines from the collection of MVCRI-Maritsa, Plovdiv using 182 SSR markers and SNP in 19 genes from different metabolic pathways. Understanding a tomato genotype's genetic diversity and population structure could help breeders to improve crop traits by identifying beneficial genetic variations within germplasm for developing more resilient and productive varieties that can withstand climate changes.

Material and Methods

Plant material

Eight Bulgarian accessions from the collection of the Maritza Vegetable Crops Research Institute, Plovdiv, Bulgaria were studied. The collection included two varieties and six advanced breeding lines (Figure 1). The variety Plovdivska karotina with a *Solanum chilense* background, is characterized by orange-colored fruits, indeterminate growth habit, and round fruit shape. The second variety, IZK Alya, derived from *Solanum pimpinellifolium*, produces red, oval-shape fruits and indeterminate growth habits. Two of the breeding lines (L975 and L984), are mutant forms with pink/red fruit color, indeterminate/determinate type and oval fruit shape. The remaining four lines (L1116, L1140, L21β, and L53β) are predominantly indeterminate, except of L53β. These lines produce fruits of distinct colors: red-violet (L1116), purple-black (L1140), yellow (L21β), and orange (L53β). Each genotype was presented by seven individual plants.

DNA extraction

DNA samples were extracted from frozen leaf tissue (250 – 300 mg) of field grown plants. A standard cethyl trimethyl ammonium bromide (CTAB) procedure was used (Murray and Thompson, 1980), with a modified extraction buffer containing 2% CTAB, 4% PvP, NaCl, Tris-HCl (pH 8.0) and EDTANa2 (pH 8.0) and 8 mol/L of LiCl as described by Todorovska et al. (2014).

SSR analysis

Primer synthesis

In total 182 published tomato SSR primers (Shirasawa and Hirakawa, 2013; Geethanjali et al., 2010; 2011; Areshchenkova and Ganal, 2002; Smulders et al., 1997; Yang et al., 2005; Shirasawa et al., 2010) (Table 1) referred to as locus-specific primers (LSPs), were synthesized with generic non-complementary nucleotide sequences tagF 5'ACGACGTTGTAAAA3' and tagR 5'CATTAAGTTCCCATTA3', respectively, at their 5' ends as described by Hayden et al. (2008). In addition, two generic tag primers, namely tagF' and tagR', with the same sequences 5'ACGACGTTGTAAAA3' and 5'CATTAAGTTCCCATTA3' were also synthesized. The tagF' primer (5'ACGACGTTGTAAAA3') was labelled at its 5' end with one of the following fluorescent dyes: FAM, ATTO565, ATTO550 and YAKIMA YELLOW (Applied Biosystems), allowing direct detection of alleles on an automated capillary sequencer (ABI3130, Applied Biosystems). All primers were synthesized by Microsynth (https://www.microsynth.com/home-ch.html).

PCR assay

All uniplex PCR reactions were performed according to Hayden et al. (2008). PCR was performed on Veriti96 Thermal Cycler (Applied Biosystems) using PCR conditions described by Todorovska et al. (2014).

Electrophoresis and visualization of SSR alleles

Electrophoresis and visualization of tomato SSRs was performed on an ABI3130 DNA analyzer (Applied Biosystems). A standardized multi-pooling procedure was used to prepare SSR products for electrophoresis. The post-PCR

Fig. 1. Tomato varieties and lines from the collection of MCRI – Plovdiv

Source: Authors' own elaboration

Table 1. Number and type of SSR markers used in the analysis

SSR markers	Databases	Optimized PCR conditions according to Todorovska et al. (2014)	
		T of annealing	Primer concentration
105 genomic SSR and 17 TGS (tomato Genome-SSR)	Sol Genomics Network (SGN); Shirasawa et al. (2010)	50/63°C	20–30 nmol/L
24 SLM markers	BAC libraries for chromosomes 6 and 12 (Geethanjali et al., 2010; Geethanjali et al., 2011)	50°C	60 nmol/L
13 TMS, 2 EST and 15 TES (tomato EST-SSR) markers	Areshchenkova and Ganal (2002); Shirasawa et al. (2010)	50/63°C	20–30 nmol/L
2 SSRs (LEMDDna; LeLe25)	Smulders et al. (1997)	50/55°C	20–30 nmol/L
4 TOM markers	Yang et al. (2005)	50°C	20–30 nmol/L

Source: Authors' own elaboration

mixing of the amplified products was performed as described by Todorovska et al. (2014).

SNP analysis in genes from different metabolic pathways

Selection of genes and synthesis of primers

DNA sequence information of 19 genes published in few databases (Shirasawa and Hirakawa, 2013; Tomato Integrated Database; dbSNP (NCBI); SolCAP) and scientific articles (Hutton et al., 2010; Francis et al., 2005) was used to design primers using the online tools PrimerDesignTool of NCBI and Primer3plus (Table 2). The sequences were selected to correspond to important genes and transcription factors involved in metabolic pathways related to the synthesis of antioxidants, vitamins, organic acids, etc. in tomato fruits. All primers excluding those for the CAPS marker LEOH200 were synthesized with generic M13F and M13R primers on their 5' ends which allowed direct sequencing of the purified PCR products.

Polymerase chain reaction

PCR reactions were performed on a Veriti Thermal cycler (Applied Biosystems) in a total volume of 20 µl including 1u High fidelity Taq DNA polymerase (Fermentas), 100 ng gDNA, 1xPCR buffer, 200 µM of each dNTP, 10 pmol of each primer and 1.5 mM $MgCl_2$. The PCR conditions were: 1 cycle of denaturation for 3 min at 94° C, 33 cycles, each including denaturation at 94° C for 40 sec, annealing at 60/62° C for 45 sec and synthesis at 72°C for 1 min.

Sequence analysis of genes

The resulting PCR fragments were separated by agarose gel electrophoresis, excised and purified by GeneJET PCR Purification Kit (Fermentas, #K0702). The fragments were sequenced on an ABI 3130xl capillary analyzer by standard protocol using complementary M13F and M13R primers. The chromatograms were visualized with the Chromas Lite 2.6 program, and the sequences were processed and analyzed with the programs: VectorNTI (Invitrogen) and GeneDoc (NRBSC). For detection of SNP in LEOH200, a PCR-RFLP approach was performed.

Data analysis

Amplified SSR fragments and the SNP in each gene were scored for the presence (1) or absence (0) of the respective bands/site in all the genotypes tested. Allele frequencies, genetic diversity index, heterozygosity and polymorphic information content for SSR and SNP markers were calculated by Power Marker 3.25 (Liu and Muse, 2005). The Nei Da distance matrices were used in Mantel test comparison and to construct the dendrograms with the un-weighted pair group method with arithmetic mean (UPGMA) module of Power Marker 3.25 (Liu and Muse, 2005).

Model-based clustering program STRUCTURE V2.3.4 (Pritchard et al., 2000) was employed to deduce the population structure of all the 8 accessions based on SSR data. Number of populations (K) was determined with a burn-in period of 50 000 and Markov Chine Monte Carlo of 2 000 000. Ten independent runs were performed for each K varying from 1 to 10 and most likely number of clusters (K) was determined by the method of Evanno et al. (2005) and the visualization of the resulting clusters was done using a docker implementation of pophelperShiny v2.1.1 (Francis, 2017).

A Mantel test using Pearson's *r*-value was also carried out for testing the relationship between distance matrices of the two types of molecular data (Mantel, 1967).

Biochemical analysis

Evaluations were performed during harvesting period in triplicates. An average sample of 20 fully ripened fruits per replication were picked from 3–4 trusses, washed and wiped. Following parameters were assessed:

– Total soluble solids were measured with a hand-held refractometer and expressed as Brix;
– Titrable acidity (TA) by using titration with NaOH;
– Ascorbic acid (Vitamin C) content – titration with 2.6-dichlorophenolindophenol (Tillman's reaction)
– lycopene and β-carotene contents were determined according to Manuelyan (1991) and expressed as mg per 100 g FW.

Results and Discussion

The knowledge of the genetic diversity and population structure of tomatoes (*S. lycopersicum* L.) is essential for both, the selection of genotypes with better agronomic performance to maximize the genetic improvement in the breeding process, maintain food security and adaptation to changing environments and for germplasm conservation. However, domestication and breeding processes have significantly reduced genetic diversity, resulting in a genetic bottleneck in cultivated varieties (Du et al., 2025).

To assess the genetic diversity in Bulgarian tomato collection consisting of 8 tomato varieties and breeding lines two types of codominant markers, SSR and SNP, were selected and applied as the most relevant for genotyping and determination of their genetic profiles.

Table 2. Name of the genes studied, chromosome location (Chr.), putative SNP location in the target gene according to the literature data, primer sequences (F and R), T of annealing (T° an.) and fragment length of PCR products

SNP marker	Name of the gene	Chr.	Localization of SNP	Forward (5′-3') primer	Reverse (5′-3') primer	Optimized T°an.	Fragment length (bp)
*Myb12 (fragment 1)**	*SlMyb12*	1	Exon 1 – Exon 2	*TCAAGAGAGGCAGATGGACT*	*TGTTACCCAAAGTTGCATGTAACT*	*59°C*	*318*
*Myb12 exon3 (fragment 2)**	*SlMyb12*	1	Exon 3	*CAGATGGTCTCTTATAGCAGAACA*	*GGCTTCCCTTGGCCTCTATA*	*59°C*	*344*
*Myb12 intr2 (fragment 3)**	*SlMyb12*	1	Intron 2	*CACAATAATTTGGTGCTC-CGATCTAAC*	*ATATTAAATTTATCACACGAACAA-CAGC*	*59°C*	*338*
*Myb12 exon4 (fragment 4)**	*SlMyb12*	1	Exon 4	*GTGATATCGAATGGCCAAGACT*	*CTAGTCATTCTAATTCCCACATTCCA*	*59°C*	*399*
*LEOH348**	LEOH348	2	Exon *	*TGTTTCCCTTCATTCATGCT*	*CCAATTGGATAAATTGGTGGT*	*52°C – 60°C*	*185*
*KetoCoA**	SGN: SGN-M22804	2	Exon 2	*ACTAACACTTATTGGACGGAAGA*	*GCTATTTGCCATACTCTATCACC*	*59°C*	*276*
*CoumCoA**	Tomatomics: KTU4-H10122	3	Exon 3	*GTTACTTGAATGACCCTGAAGCTA*	*GCGCCACTTGAAATCCTTTGTA*	*60°C*	*153*
*Sulfoprot**	Tomatomics: KTU4-H10742	3	Exon*	*ATCCCTTTGCTCACAACTGGT*	*GGTGTTGGATTTGTTGGGTGG*	*63°C*	*353*
*Methyltrans**	SGN: SGN-M24798	3	Exon 1	*TAGCGTCTCAACTCCCTTGT*	*ACACCATCGGCATTCTTAGT*	*59°C*	*181*
*LycopeneC1**	SGN: SGN-M17867	4	Exon*	*CTTGTACCTGAGACCAAAAAGG*	*AGTCCATAGCCTCAAATTCATC*	*59°C*	*229*
*GeranylPyr**	Kazusa: 14039_273	4	Exon*	*AGCTGGACAAGTAGCGGATT*	*AGCTCCGACGATGACTTTG*	*60.5°C*	240
LEOH200	LEOH200	6	Exon*	*GGGTTTATGTTGGTGATATGGTG*	*TCAGCAGCTAAAAGTCGAACC*	*52°C– 60°C*	176
*UDPSugar**	SGN: SGN-M23456	6	Exon 1	*CCTCTAACCTCTCCATCTTCC*	*CACTCTGCTTCTGTCCTTCA*	*59°C*	207
*MannoseIso**	SGN: SGN-M24624	6	Exon 5	*TTCTTCCACAAAACTCAACTACTG*	*ACTCAAACAACCTGCTGCTAACT*	*59°C*	213
*HydroCoA**	Tomatomics: KTU4-E45581	7	Exon 2	*AAAATCGCGAGTCCAGTACCA*	*AAAGCCTTGACCTTCCGTCG*	*68°C*	201
*CarotIsomer**	SGN: SGN-M24984	10	Exon 1	*GCAAACCAAGATACAATAAACAGA*	*CACTCTCATTTCCTTCTACATCAC*	*59°C*	213
Antocyanin 1(Ant1)	Anthocyanin 1	10	Full gene sequence	GGGAGTGAGAAAAGGTTCATGG ATTGCTGGTAGACTTCCCGG	ACTTGCGTCGTTGAGGTTTT CTCCTTGTTGCATGGGTGG	*59°C*	933
*Fru1,6bis**	SGN: SGN-M25463	12	Exon 1	*AGAAGAAAGCAGAGCGATGGA*	*TTGCAGCCAAGAACAATATGAC*	*65°C*	150
FlavaHydro	*Flavanone 3-hydroxy-lase-like protein*	3	Exon 3	*TGCAGGGAAATTCGTCAACTC*	*CAAAGGCGTCAGGTTGAGGT*	60°C	280
RecProt	*Receptor expression-enhancing protein 3*	4	Exon 5	*CATCACTAAATCGCAGGTCATC*	*CTTCCTCCAAGGCTGTCTCA*	*60°C*	363
Sec14-Prot	*SEC14-like protein*	11	Exon 4	*TGACAAAGACGGAAGACCAG*	*CACTCCTTGCACATCCAAAA*	*60°C*	205
CathepsinCys	Cathepsin B-like cysteine proteinase	2	Exon 4	*GGGAAAATCTTGGGGCATGGA*	*GGCAGAGGCCTTGTAAAACA*	*60°C*	323

*All primers were synthesized with addition of universal M13 tails on their 5'ends. *The lack of exon numbering indicates the presence of a single exon.
Source: Authors' own elaboration

Genetic diversity assessment based on SSR markers

SSR analysis was performed with 182 genomic SSR, TGS, EST, TES, SLM, TOM, TMS, LEMDDna and LeLe25 markers. They were selected from different databases and literature data (Table 1) to represent different genetic loci across the all 12 tomato chromosomes. Of the applied 182 SSRs, 162 were used in a previous genetic diversity assessment of the same collection (Todorovska et al., 2014). The lowest number of markers in this study was on chromosome 9, while the highest number of markers used (between 17 and 21 SSRs) was on chromosomes 1, 2, 3, 4, 6 and 12.

The study showed that of the used SSR markers, 48 % (in total 96 SSRs) were polymorphic in the Bulgarian tomato collection consisting of 8 modern tomato varieties and

breeding lines. A total of 258 alleles were detected with a mean of 2.69 alleles per locus. These data are comparable with the data observed by other authors in the cultivated tomato using SSR markers (Pidigam et al., 2021), but higher than those observed by Khan et al. (2024). Seventeen of the studied SSR loci had between four and six alleles (Table 3, Appendix). Twenty-six of the polymorphic SSR markers produced 3 alleles per locus while the remaining ones (53) produced only 2 alleles per locus. Most of the observed alleles were found in variety IZK Alya (cherry type), which is consistent with the previous investigations reporting that typically cherry tomato accessions showed a large genetic diversity in comparison to large cultivated ones (Todorovska et al., 2014; Bauchet and Causse, 2012).

PIC value of the polymorphic SSR markers ranged from 0.11 to 0.78 with an average of 0.375, which is similar to that observed in the earlier reported PIC values of 0.31 (Benor et al., 2008; Khan et al., 2024), 0.37 (He and Yu, 2003), 0.39 (Frary et al., 2005), 0.40 (Bredemeijer et al., 2002), 0.45 (Glogovac et al., 2013) in different genetic diversity studies in tomato, although the number of genotypes in the studied Bulgarian collection is much smaller. Among the polymorphic markers used in this study, 27% showed PIC value higher than 0.5. SML6 – 7 recorded the highest PIC with 0.78 followed by TMS 65, SLM 6 – 15 and SLM 6 – 48 having PIC values greater than 0.7 values. This indicates that these primers are expected to be effective for revealing the genetic differences within large collections of tomato varieties and wild relatives, making them a valuable tool for breeders and researchers in assessment and utilization of the genetic variation for future improvement and germplasm management in tomatoes. Our study reaffirmed also the utility of SSR111 (Table 3, Appendix) in gene diversity studies (Frary et al., 2005; Chen et al., 2009; Glogovac et al., 2013).

The gene diversity (GD) estimates varied between 0.1099 (SSR 344) and 0.7109 (SLM 6 – 7) with a mean 0.369 (Table 3, Appendix), a value comparable with other studies in cultivated tomato (Todorovska et al., 2014; Bredemeijer et al., 2002; He and Yu, 2003), but higher than the reported in Bolivian wild and cultivated tomato (He = 0.067) (Villanueva-Gutierrez et al., 2022). The level of heterozygosity is limited, with a mean value of 0.0377. The highest level of heterozygosity was observed in loci TMS22 and TMS63 (Table 3, Appendix). The observed GD and Ho values confirmed that modern breeding in tomatoes is mostly oriented to selection of specific phenotypes with better agronomic and nutritional traits thus limiting the genetic variation of the population – a bottleneck that limits future breeding gains and necessitates exploring wild relatives and traditional landraces for new genetic resources.

SNP polymorphisms in genes from different metabolic pathways in tomato

Single nucleotide polymorphisms are the most abundant source of variation in both the intragenic and the intergenic regions in the tomato genome, making them a valuable foundation for developing molecular markers to differentiate closely related tomato genotypes, determine gene diversity

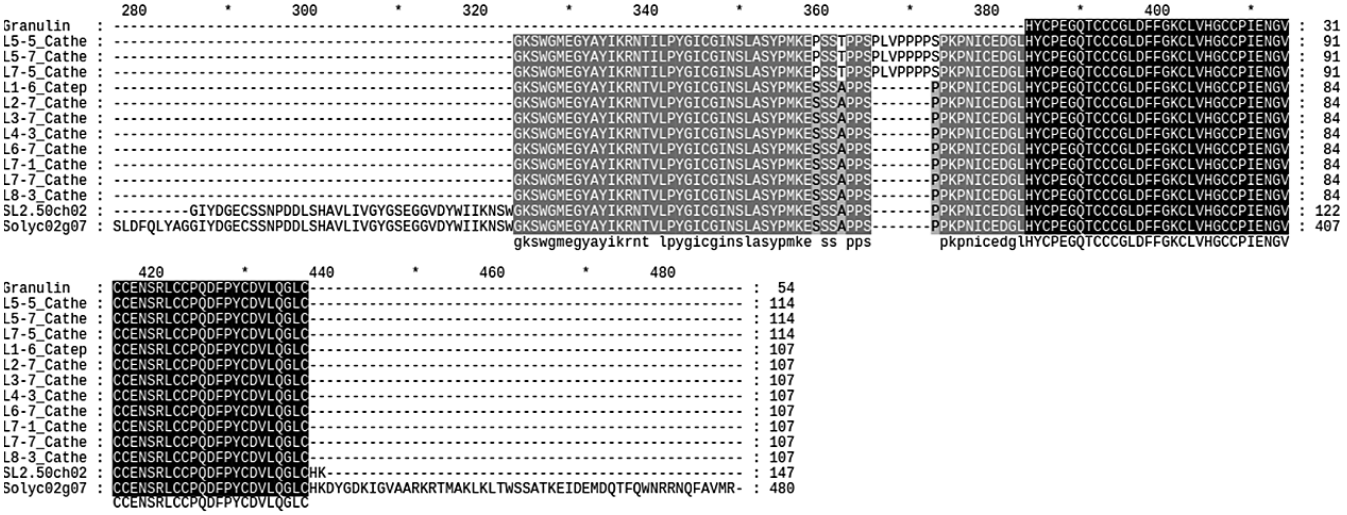

Fig. 2. SNP polymorphisms (nonsynonymous SNPs) in *Cathepsin B-like cysteine proteinase* (upstream to the Granulin domain) leading to amino acid substitutions and an indel representing the insertion of 21 nucleotides (additional 7 amino acids) in all plants of cv. IZK Alya (line L5) and in one of L975 (line L7).

Source: Authors' own elaboration

in tomato collections (Corrado et al., 2013; Shirasawa et al., 2010) and mapping important traits (Adedze et al., 2025).

In this study, SNP analysis was performed using primers amplifying different parts of 19 genes involved in various metabolic pathways in tomato (Table 2). In total 34 SNPs were observed mostly in the exons of 13 of the studied 19 genes, some of which leading to amino acid substitutions. Such substitutions were observed in the exons of genes encoding for *Flavanone 3-hydroxylase-like protein*, *Receptor expression-enhancing protein 3*, *SEC14-like protein* and *Cathepsin B-like cysteine proteinase*. The highest number of SNPs (in total 5) was observed in the exon 3 of the gene for *Cathepsin B-like cysteine proteinase.* In this gene an indel representing an insertion of additional 21 nucleotides except of SNP leading to 3 amino acid substitutions was observed near to the granulin domain of this gene (Figure 2).

Regarding the mean number of alleles per site, similar results have been reported by Chen et al. (2025) in a study of gene diversity in 484 tomato accessions using 48 pairs of SNP markers covering the entire tomato chromosomes.

The mean gene diversity and PIC based on the observed SNPs was moderate – 0.3161, 0.2895, respectively (Table 4), but similar to those obtained with the applied SSR markers, thus confirming the informativeness of the targeted SNP markers. The observed mean gene diversity and mean PIC values in this study were higher than those reported by Alatawi et al. (2025) (mean GD = 0.12; mean PIC = 0.09), who analyzed a USDA-GRIN collection of 276 accessions using SNPs generated through genotyping by sequencing (GBS). However, the values were lower than those reported by Xu et al. (2024) (mean GD = 0.49; mean PIC = 0.37) in 418 modern tomato varieties analyzed using genome-wide SNP markers, and by Chen et al. (2025) (GD = 0.428) in 484 self-compatible and homozygous cultivated tomato accessions used for the construction of a core collection in China. In addition, the level of Ho was lower in our collection (0.0515) in contradiction to that reported by the above-mentioned authors (mean Ho = 0.08).

Similar to SSR markers the cherry-type tomato (cv. Alya) showed a higher number of SNPs in comparison to other inbred tomato varieties and lines. Such observation has been also reported by Shirasawa et al. (2010), who identified higher levels of genetic variation in cherry-type of tomato than in the inbred tomato lines, with 310.1 (23.2%) SNPs on average. This showed that the SNP markers in tomatoes are useful tools not only for genetic diversity studies but for QTL mapping of important traits like fruit quality, biotic and abiotic stress tolerance.

Statistically similar genetic distance matrices based on SSR and SNP markers as confirmed by the Mantel test

Table 4. Genetic diversity assessment of Bulgarian tomato as estimated by the major allele frequency, allele number, gene diversity (GD or He), heterozygosity (Ho) and polymorphic information content of SNP markers (PIC)

Marker	Major Allele Frequency	Allele No	Gene Diversity	Heterozygosity	PIC
LEOH348 (SNP)	0.750	2	0.3281	0	0.3047
Myb12 (intron1,2)	0.625	2	0.4102	0	0.3589
Fru1,61Is	0.625	2	0.4102	0	0.3589
Sulfoprot	0.750	2	0.3281	0	0.3047
CoumCoA	0.500	2	0.4375	0	0.3750
CarotIsomer_Heteroz	0.875	2	0.2061	0.25	0.1948
KetoCoA	0.875	2	0.1914	0	0.1948
Methyltrans	0.750	2	0.3281	0	0.3047
MannoseIso	0.750	2	0.3281	0	0.3047
Flavo_Hydro	0.625	2	0.4102	0	0.3589
Sec14	0.500	2	0.4375	0	0.3750
RecProt	0.875	2	0.1914	0	0.1948
Cathepsin 1_1	0.8125	2	0.2736	0.125	0.2582
Cathepsin 1_2	0.8125	2	0.2736	0.125	0.2582
Cathepsin 1_3	0,8125	2	0.2736	0.125	0.2582
Cathepsin 1_4_InDel	0.8125	2	0.2736	0.125	0.2582
Cathepsin 1_5	0.8125	2	0.2736	0.125	0.2582
Total		34			
Mean	0.7390	2	0.3161	0.0515	0.2895

Source: Authors' own elaboration

($p < 0.0001$) has been also reported by Ramirez-Ramirez et al. (2024) in *Theobroma cacao* L.

Genetic diversity of Bulgarian tomato based on combined SSR and SNP markers

The genetic analysis based on combined data from microsatellite and SNP markers yielded a total of 252 different alleles across all analyzed loci, with a mean number of alleles/locus – 2.584. The mean GD, Ho and PIC were 0.3670, 0.0398 and 0.3629, respectively, indicating a moderate level of genetic variation in Bulgarian tomato despite the use of two types of codominant markers (data not shown). This could be explained with the lower number of the applied polymorphic SNP markers and the fact that comparing to SSR markers, SNPs are mostly bi-allelic, the level of polymorphism is much lower and therefore, higher number of loci sufficiently polymorphic might be necessary to reach the same power as multi-allelic SSR loci (Guichoux et al., 2011). However, our study showed that a relatively low number of highly informative SNPs can potentially give similar genetic resolution as randomly chosen multi-allelic SSRs similar to Kaiser et al. (2016). To enhance gene diversity, phylogenetic and population studies in tomato, high-throughput genotyping using the SNP chips in tomato collection with a large number of polymorphic SNP markers is necessary.

Genetic structure

Genetic relationship between the studied accessions was evaluated using cluster analysis. The SSR based UPGMA tree clearly separated the cherry-type IZK Alya as an outgroup. L1140 and L1116 also showed to be genetically divergent, branching successively outside of the main cluster. The latter consisted of two subclusters, formed by grouping L21β and Plovdivska karotina in the first one, and L984, L975 and L53β in the second one (Figure 3A). The SNP based clustering also separated IZK Alya as an outgroup, however contrary to the SSR dendrogram the next most divergent genotype was L975. The main cluster consisted of two subclusters, the first one formed by L53β and L1140, and the other by the pairs of L21β, L984, L1116 and Plovdivska karotina (Figure 3B). The combined dendrogram (SSR and SNP) showed grouping of the studied genotypes which is more consistent with weight of the fruits.

The Mantel test revealed good correlation between the SSR and SNP genetic distance matrices (0.7097), thus confirming that the information generated by the two sets of markers is highly similar ($p < 0.03$). This indicates that both types of markers provide reliable genetic information and they are appropriate tools for further genetic analyses of Bulgarian tomato germplasm.

The genetic distances among the genotypes based on SSR and SNP markers showed the largest pairwise distances between IZK Alya and L984 (0.77876), followed by IZK Alya and L1116 (0.75114), and IZK Alya and Plovdivska karotina (0.74595). The lowest pairwise distance (0.19728) was observed between Plovdivska karotina and L21β (Table 5). The dendrogram constructed using the combined data confirmed (Figure 3C) the estimated genetic distances between

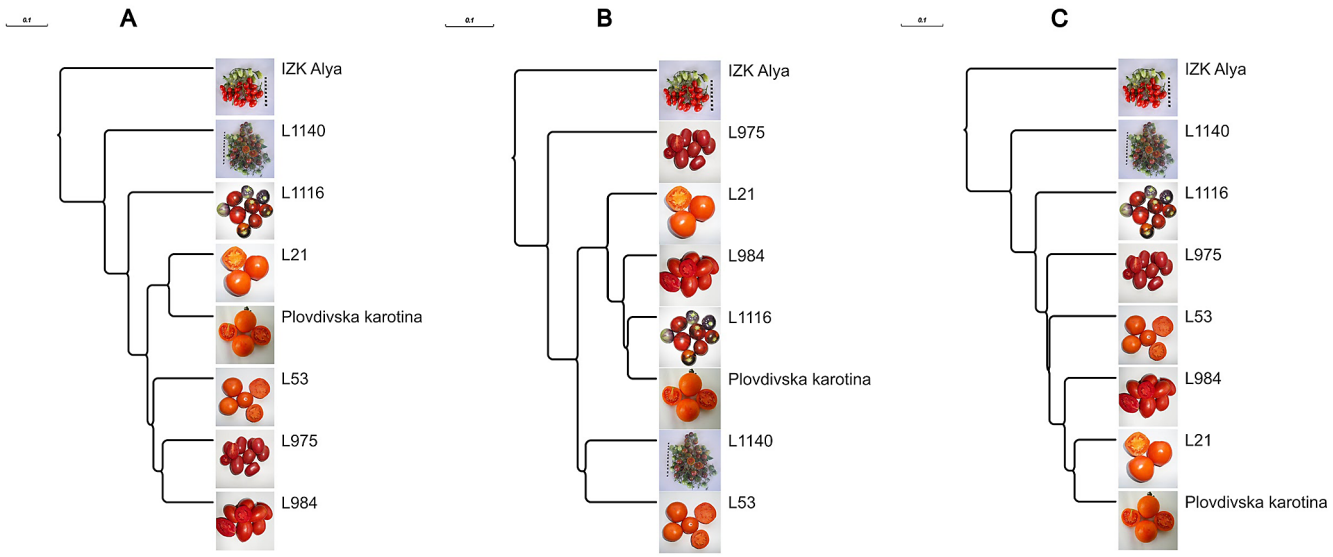

Fig. 3. UPGMA dendrograms. A) SSR markers; B) SNP markers; C) combined data (SSRs & SNPs)
Source: Authors' own elaboration

Table 5. Genetic distances between tomato genotypes based on SSR and SNP markers

Genetic distances	IZK Alya	L1116	L1140	L21β	L53β	L975	L984	P. karotina
IZK Alya	0							
L1116	0.7511	0						
L1140	0.6140	0.5130	0					
L21β	0.6929	0.3565	0.5052	0				
L53β	0.7017	0.3972	0.4521	0.3363	0			
L975	0.7324	0.4721	0.5004	0.3492	0.3404	0		
L984	0.7788	0.3633	0.5343	0.2504	0.2795	0.2659	0	
P. karotina	0.7459	0.3315	0.5078	0.1973	0.3389	0.3607	0.2379	0

Source: Authors' own elaboration

the studied tomato genotypes and showed that the IZK Alya is the most distant genotype similarly to the dendrograms based on SSR and SNP markers separately.

The genetic structure of the studied sample was also evaluated using a model-based method. The STRUCTURE analysis was conducted with the SSR markers alone and in combination with SNP data. The most probable cluster number was defined using ΔK value Evanno et al. (2005). Structure analysis, using SSR data alone, grouped the genotypes into 2 populations (Figure 4A). At *K*=2 IZK Alya formed a separate cluster, while the remaining genotypes were grouped together as a distinct population, with L1140 showing an admixed ancestry (Figure 4B). At *K*=3 L1140 was also separated in a distinct group of its own, and L1116 was defined as admixed between the latter population and the one where the rest of the genotypes, excluding IZK Alya, were grouped. These observations are in line with the results of UPGMA clustering, where these three genotypes formed distinct branches from the main cluster in Figure 3A. The combined data results show 3 as most probable number of populations (Figure 4C), once again the cherry type IZK Alya and L1140 separated in one-member genetically distinct groups,

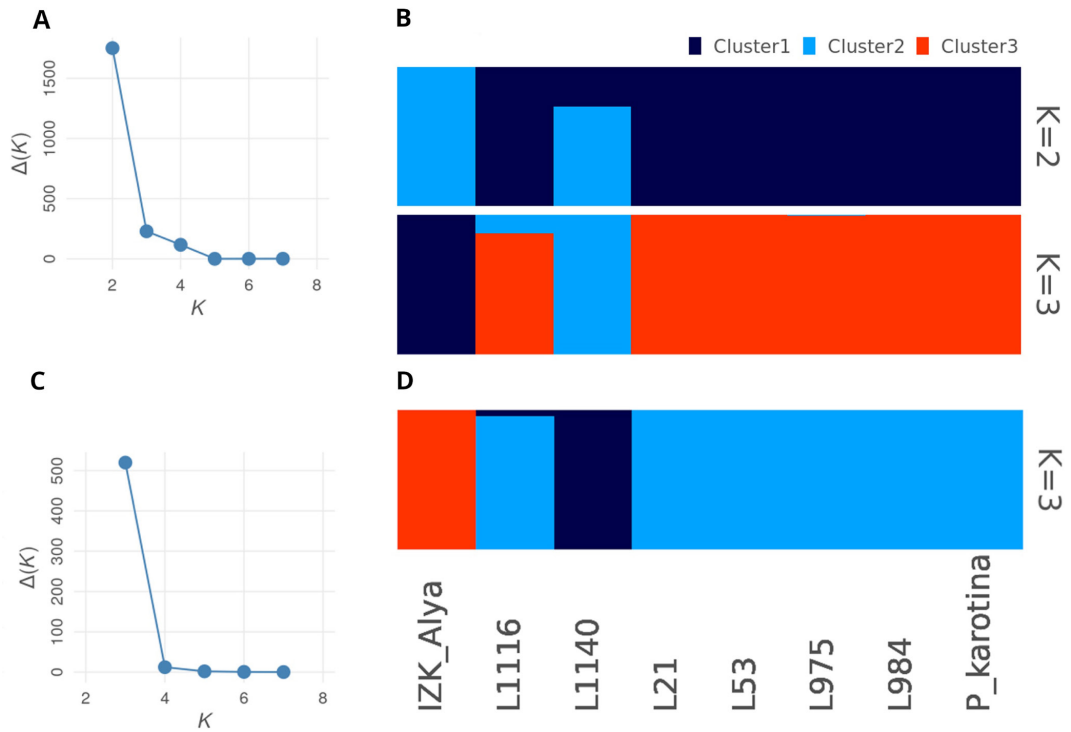

Fig. 4. STRUCTURE analysis of tomato genotypes based on SSR markers (A and B); SSR and SNP markers (C and D)
Source: Authors' own elaboration

and L1116 showed to be a result of admix between the latter and the population grouping the remaining five accessions (Figure 4D).

Biochemical analysis

Tomato quality parameters, crucial for processing, consumers, and breeders, include fruit color and chemical composition, specifically dry matter (brix), ascorbic acid, titratable organic acids (TOA), total pigments, lycopene and β-carotene, and sugars, along with their ratio in the fruit (Viskelis et al., 2015). Epidemiological studies showed that regular consumption of tomato products acts as a functional food, offering protection against chronic diseases like cancer and inflammatory conditions, while promoting health in neurodegenerative, metabolic, and skeletal disease prevention (Crupi et al., 2023). These health benefits are attributed to tomato's bioactive compounds, particularly the antioxidants lycopene and β-carotene, which reduce oxidative stress and modulate inflammatory pathways, though the exact mechanisms are still being investigated.

Biochemical analysis of the fruits of the 8 genotypes analyzed here (Table 6) showed that the varieties IZK Alya and Plovdivska karotina are characterized with the highest content of ascorbic acid and total pigments. The powerful antioxidant carotenoid lycopene is the most abundant in both, IZK Alya and L984, while Plovdivska karotina and L21β are characterized with the highest amount of β-carotene. Among all tomato genotypes the highest brix content was established in IZK Alya. The data showed that IZK Alya could be an important source for brix, ascorbic acid, total pigments and lycopene while Plovdivska karotina for ascorbic acid, total pigments and β-carotene.

The obtained information is of importance for further breeding programs directed to improvement of fruit quality traits, inclusion of these genotypes in human diet as well as for farmers and processing industry in Bulgaria.

Conclusion

To our knowledge, the present study is the first one employing a large number of SSR and SNP markers for characterization of Bulgarian tomato. It showed that both marker types are informative and can be successfully used for discrimination of the selected Bulgarian tomato accessions. Among the analyzed tomato genotypes, the variety IZK Alya and the breeding lines L1140 and L1116 possess distinct genetic makeups making them valuable allele sources for expanding allelic diversity in cultivated tomatoes.

The research demonstrated that a small, targeted number of SNP markers (17) could provide the same discriminatory power as a much larger number of SSR markers used (182), thus highlighting the efficiency of targeted SNP analysis for genetic diversity and genome-wide association studies (GWAS) for agronomic, fruit quality, and other traits related to biotic and abiotic stress tolerance in tomatoes.

This study contributes to a comprehensive understanding of the genetic diversity within the Bulgarian tomato accessions and provides essential knowledge for their effective utilization in breeding programs for development of new resilient varieties with improved nutrition and yield traits. It can serve also as a valuable foundation for better management of tomato germplasm resources.

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Table 6. Biochemical indicators of fruits of tomato varieties and lines

Variety, Breeding lines	TSS, (◦Brix)		Ascorbic acid (mg/100gFW)		Titratable organic acids (% citric acid)		Total pigments (mg %)		Lycopene (mg %)		β-carotene (mg%)	
	$\bar{x}$	±sd	$\bar{x}$	±sd	$\bar{x}$	±sd	$\bar{x}$	±sd	$\bar{x}$	±sd	$\bar{x}$	±sd
P. karotina	5.1	0.9	41.47	5.11	0.36	0.01	9.33	1.09	4.47	0.79	4.24	0.73
L21β	7.1	0.5	24.97	3.37	0.42	0.02	4.83	0.69	–		4.59	0.95
L1116	5.4	0.1	32.87	10.54	0.41	0.03	7.07	0.47	6.14	0.64	0.44	0.19
L1140	7.3	0.5	33.40	4.51	0.53	0.04	6.06	0.83	4.16	1.26	1.18	0.56
IZK Alya	8.5	0.2	45.13	11.32	0.59	0.02	9.52	1.01	8.81	0.56	0.18	0.25
L984	4.3	0.1	31.60	2.00	0.26	0.02	8.97	1.09	8.37	1.39	–	
L975	5.4	0.2	29.27	1.44	0.25	0.01	8.48	1.41	7.76	0.95	–	
L53β	4.5	0.2	25.73	6.82	0.32	0.05	4.65	0.49	1.39	0.30	3.19	0.344

Source: Authors' own elaboration

Author contributions

Elena G. Todorovska (E. G. T.) acquired funding, conceptualized and designed the experiment, performed molecular and data analyses, prepared the original draft, and reviewed drafts, approved the final draft. Nikolai K. Christov (N. K. C.) analyzed data, prepared tables and figures, prepared and reviewed drafts of the paper and approved the final draft. Daniela Ganeva (D. G.) described and contributed the plant material, reviewed drafts of paper and approved the final draft. Stanislava Grozeva (S. G.) and Ivanka Tringovska (I. T.) performed biochemical analyses, reviewed drafts of paper and approved the final draft. Stefan Tsonev (T. S.) analyzed data, prepared and reviewed all drafts of the paper and approved the final draft. Nasya Tomlekova (N. T.) acquired additional funding, reviewed drafts of the paper and approved the final draft. All authors have read and agreed to the published version of the manuscript.

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Declarations of conflict of interest

The authors declare that there is no conflict of interest.

Appendix (Table 3). Genetic diversity assessment of Bulgarian tomato as estimated by the allele number, gene diversity (GD or He), heterozygosity (Ho) and polymorphic information content of SSR markers (PIC)

Marker	Allele No	Gene Diversity (He)	Heterozygosity (Ho)	PIC
SSR 5	2	0.1914	0	0.1948
SSR 13	2	0.1914	0	0.1948
SSR 14	2	0.1914	0	0.1948
SSR 19	2	0.1914	0	0.1948
SSR 20	2	0.4375	0	0.375
SSR 22	3	0.3555	0	0.3706
SSR 26	2	0.3281	0	0.3047
SSR 32	2	0.3281	0	0.3047
SSR 43	2	0.1914	0	0.1948
SSR 45	3	0.5195	0	0.5112
SSR 47	2	0.4375	0	0.375
SSR 50	3	0.3555	0	0.3706
SSR 46	2	0.1914	0	0.1948
SSR 65	2	0.1914	0	0.1948
SSR 63	4	0.6016	0	0.6299
SSR 69	2	0.1914	0	0.1948
SSR 70	4	0.6153	0.125	0.6445
SSR 74	2	0.1914	0	0.1948
SSR 81	2	0.3281	0	0.3047
SSR 92	2	0.3281	0	0.3047
SSR 95	3	0.2053	0.125	0.2146
SSR 96	2	0.2736	0.125	0.2583
SSR 103	2	0.1914	0	0.1948
SSR 111	3	0.5469	0	0.5547
SSR 115	2	0.3281	0	0.3047
SSR 128	4	0.6279	1	0.5815
SSR 134	3	0.4648	0	0.4683
SSR 139	2	0.1914	0	0.1948
SSR 223	2	0.1914	0	0.1948

Appendix (Table 3). Continued

SSR 231	2	0.4102	0	0.3589
SSR 241	2	0.1914	0	0.1948
SSR 244	2	0.1914	0	0.1948
SSR 248	3	0.5742	0	0.5815
SSR 266	3	0.5469	0	0.5547
SSR 276	3	0.2873	0.125	0.2944
SSR 289	2	0.1914	0	0.1948
SSR 310	2	0.3281	0	0.3047
SSR 318	3	0.5742	0	0.5815
SSR 326	2	0.1914	0	0.1948
SSR 333	3	0.5195	0	0.5112
SSR 344	2	0.1099	0.125	0.1103
SSR 349	3	0.5195	0	0.5112
SSR 350	2	0.3829	0.125	0.3374
SSR 383	2	0.4375	0	0.375
SSR 450	2	0.3281	0	0.3047
SSR 565	2	0.1914	0	0.1948
SSR 578	2	0.3281	0	0.3047
SSR 580	2	0.1914	0	0.1948
SSR 598	2	0.3281	0	0.3047
SSR 601	2	0.1914	0	0.1948
SSR 605	2	0.1914	0	0.1948
TGS 914	2	0.4102	0	0.3589
TGS 959	2	0.4102	0	0.3589
TGS 1606	2	0.1914	0	0.1948
EST 253712	2	0.4375	0	0.375
TES 1711	2	0.1914	0	0.1948
TES 1276	2	0.3281	0	0.3047
EST 245053	2	0.1914	0	0.1948
TMS 9	3	0.5742	0	0.5815
TMS 22	4	0.6468	0.875	0.6237
TMS 26	2	0.3281	0	0.3047
TMS 33	3	0.547	0.125	0.5439
TMS 42	3	0.3555	0	0.3706
TMS 59	2	0.3281	0	0.3047
TMS 63	4	0.6718	0.625	0.675
TMS 65	5	0.6562	0	0.7119
TOM 184	2	0.3281	0	0.3047
TOM 196	3	0.5742	0	0.5815
TOM 210	2	0.4102	0	0.3589
TOM 236	4	0.6289	0	0.6675
SLM 6-7	6	0.7109	0	0.7861
SLM 6-11	4	0.5742	0	0.605
SLM 6-12	3	0.4648	0	0.4683
SLM 6-14	3	0.3554	0	0.3706
SLM 6-15	5	0.6563	0	0.7119
SLM 6-17	3	0.4648	0	0.4683
SLM 6-18	2	0.1914	0	0.1948

Appendix (Table 3). Continued

SLM 6-24	3	0.3555	0	0.3706
SLM 6-25	4	0.6016	0	0.6299
SLM 6-48	5	0.6562	0	0.7119
SLM 6-56	4	0.4922	0	0.5244
SLM 6-57	3	0.4648	0	0.4683
SLM 12-5	2	0.1914	0	0.1948
SLM 12-8	2	0.1914	0	0.1948
SLM 12-10	3	0.4648	0	0.4683
SLM 12-12	4	0.547	0.125	0.5725
SLM 12-14	3	0.3555	0	0.3706
SLM 12-15	2	0.1914	0	0.1948
SLM 12-16	3	0.3555	0	0.3706
SLM 12-18	3	0.3555	0	0.3706
SLM 12-28	2	0.1914	0	0.1948
SLM 12-31	4	0.6289	0	0.6675
SLM 12-34	2	0.1914	0	0.1948
SLM 12-41	3	0.3555	0	0.3706
LeLe25	5	0.6562	0	0.7119
LEMDDNa	4	0.6427	0.125	0.675
Total	258			
Mean	2.69	0.369	0.0377	0.375

Source: Authors' own elaboration

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