BIHAREAN BIOLOGIST 15 (2): 142-148
Article No.: e214501

http://biozoojournals.ro/bihbiol/index.html

5th Balkan Scientific Conference on Biology (BalkanBio'21),
Plovdidv (online), Bulgaria, 15-16 April 2021

# Characterization of tomato genotypes by simple sequence repeats (SSR) molecular markers

Sibel AZIZ[1,3], Yaprak KANTOGLU[2], Nasya TOMLEKOVA[1]*, Teodora STAYKOVA[3], Daniela GANEVA[1] and Fatma SARSU[4]

1. Breeding Department, Maritsa Vegetable Crops Research Institute, 32 Brezovsko shoose, 4003 Plovdiv, Bulgaria.
2. Nuclear Energy Research Institute, Turkish Energy Nuclear and Mineral Research Agency, 06983 Kahramankazan, Ankara Turkey.
3. Department of Developmental Biology, Faculty of Biology, University of Plovdiv "Paisii Hilendarski", 2 Todor Samodumov, 4000 Plovdiv, Bulgaria.
4. Section of Plant Breeding and Genetics, International Atomic Energy Agency 5 Wagramer Strasse, 1400 Vienna, Austria.
* Corresponding author, N. Tomlekova, E-mail: nasia.tomlekova@gmail.com

Received: 31. January 2021 / Accepted: 25. May 2021 / Available online: 05 December 2021 / Printed: December 2021

**Abstract.**Microsatellites are highly polymorphic regions containing gene loci represented by multiple alleles of different lengths and nucleotide sequences. The purpose of this study was to identify reproducible specific molecular profiles of tomato varieties and $F_1$ hybrids from the collection of the Maritsa Vegetable Crops Research Institute, Bulgaria, and of mutant lines from the Nuclear Energy Research Institute, Turkey, by detecting polymorphic microsatellite loci as markers. The study included five varieties, four $F_1$ hybrids Bulgarian tomato, five Turkish mutant lines, and an initial landrace analysed with 19 SSR markers located in different chromosomes of the tomato genome at a distance of 6.0 cM to 146.0 cM. Fifteen microsatellite markers were used to generate polymorphic profiles. The number of bands per microsatellite locus ranged from one to eight, with a total of 62 bands among the markers analysed. The average number of SSR alleles per locus was 3.26. The dendrogram constructed with the values of genetic distance showed that all fifteen genotypes are grouped into two major clusters. Twelve form one main cluster with subclusters, while the rest of them form the other cluster. The results of the present study complement the data on the genetic heterogeneity of tomatoes and could be useful for tomato breeding programs.

**Key words**: SSR, microsatellite markers, tomato varieties, $F_1$ hybrids, mutant lines.

## Introduction

Tomato is one of the most widely cultivated crops in the world (Naika et al. 2005). These are economically important vegetable crop and favored by many populations in Bulgaria and Turkey. The tomato plant (*Solanum lycopersicum* L.) has a relatively compact genome among the *Solanaceae* family, characterized by its diploidy (2n = 2X = 24; Shirasawa et al. 2010). The tomato has emerged as one of the most studied plant species in both conventional breeding and molecular identification research. The fruits appeal to our senses because of their rich array of flavors, shapes, sizes, and colors. Several quality parameters are particularly important for tomato production and consumer requirements (Zorb et al. 2020). Tomatoes are a rich source of vitamins, minerals, phenolic content, flavonoid groups, dietary fibers, proteins, and a large number of antioxidant compounds, helping prevent many types of cancer (Tilahun et al. 2017).

Tomato hybrids are the primary source of tomato cultivation. These resources have narrow genetic bases because of several population bottlenecks in the natural, artificial selection and forms of founder events during the evolution and domestication of modern cultivars (Rick et al. 1976; Alzahib et al. 2021).

In Bulgaria, the accessions of cultivated and wild species of tomatoes are maintained in the gene banks of the Institute of Plant Genetic Resources (IPGR), Sadovo and the Maritsa Vegetable Crops Research Institute (MVCRI), Plovdiv (Todorovska et al. 2014). Cultivated tomatoes have a narrow genetic base due to continuous selection and genetic improvement (El-Mansy et al. 2021).

Variations in karyotype parameters, such as chromosome number, structure, and genome size data, have been shown to have an important role in the genetic diversity of the species (El-Mansy et al. 2021). Tomatoes have the same importance in Turkey, and there are many research programs for the development and characterization of new tomato varieties (Anonymous 2020).

In breeding studies, the selection efficiency in tomatoes is improved by the use of molecular markers (Foolad 2007). *S. lycopersicum* L. is a remarkable model system for genetic studies in plants (Frary et al. 2005). It is one of the first plants with an available molecular map based on high-density DNA (Bernatzky and Tanskley 1986, Tanskley et al. 1992, Frary et al. 2005). The presence of a genetic map containing highly reproducible, locus-specific codominant PCR-based markers facilitates molecular studies in tomatoes such as genotyping, quantitative trait locus (QTL) analysis, and marker-assisted selection (MAS) in research and selection programs (Frary et al. 2005). Microsatellites are short tandem repeats scattered throughout the genome of higher organisms. Simple sequence repeat (SSR) markers are often preferred for molecular selection due to their high reproducibility, specificity, and easy automation. In the SSR technique, primers complementary to the conserved regions that flank a microsatellite are used to amplify the repeat (Frary et al. 2005). Many SSR markers have been identified in tomatoes (Smulders et al. 1997, He et al. 2003, Foolad 2007), but only a limited number of SSR markers have been mapped (Areshchenkova and Ganal 2002). As early as 1997, Smulders and co-authors (1997), and later, Bredemeijer (2002) and He et al. (2003), confirmed the significant role of SSR markers in the study of genetic diversity and variability of the genus *Solanum*. Today, there are several databases in which information on the genome of *S. lycopersicum* L. can be found, but the largest and the one that was used in this study is the Sol Genomics Network Database. Genetic libraries, which consist of marker-defined genomic regions taken from wild species with their introgression onto the

background of elite crop lines, provide plant breeders with material and knowledge that may help to improve the agricultural traits of modern tomato cultivars (Zamir 2001, Kulus 2018). The objective of this study was to identify fifteen accessions of the tomato collection, including Bulgarian varieties, $F_1$ hybrids, and mutant lines from Turkey, using SSR markers to assess the extent of genetic diversity.

**Material and Methods**

Plant material

Fifteen tomato accessions: five Bulgarian varieties, four $F_1$ hybrids, five Turkish mutant lines (at $M_7$ generation), and an initial landrace (Ayaş) were tested. Of those included in the present study, seven Bulgarian genotypes (IZK Niki D $F_1$, Rozovo sartse, Aleno sartse, Kopnezh $F_1$, Vodoley $F_1$ , IZK Olimp $F_1$, Ideal), five mutant lines at $M_7$ generation (No. 21, No. 22, No. 50, No. 55, No. 96), and their initial (Ayaş) originate from the cultivated species *S. lycopersicum* L. One Bulgarian variety, Plovdivska karotina, was obtained from interspecific hybridization between the cultivated species (*S. lycopersicum* L.) and a wild species (*S. chillense* L.). The Bulgarian variety IZK Alya originates from a wild species (*S. pimpinellifolium* L.). The tomato varieties and $F_1$ hybrids were developed at the Vegetable Crops Research Institute, Bulgaria, and mutant lines were developed at the Nuclear Energy Research Institute, Turkey (Table 1). The genotype Plovdivska karotina has featuring a unique combination high content of three components with antioxidant effect - vitamin C, lycopene and β-carotene (Ganeva et al.2016). The inbred mutant lines No. 21, No. 22 and No. 96 have also higher lycopene, β-carotene and vitamin C levels, compared to initial landrace. Additionally the induced mutant lines No. 22 and No. 96 possess resistance to the pathogen *Fusarium oxysporum* f. *lycopersicum,* which causes bacterial disease in tomatoes (Kantoglu et al. unpublished data).

Selected mutant lines were obtained from the mutation breeding program. In this breeding program, 1500 seeds of the landrace Ayaş were irradiated at $EMD_{50}$ (150 Gy) with a $^{60}Co$ irradiator source, at the dose rate of 350 Gy/h. Mutation breeding cycles were carried out to yield 15,000 individual mutant plants. Inbred mutant lines were selected from this mutant population, and the mutation frequency was calculated as 4.7%. Each mutant line was self-pollinated according to the breeding procedure (Kantoğlu et al. 2014).

Bioinformatics review

A bioinformatics review was performed to select SSR markers for use in this study. The criteria used to select microsatellite markers from the tomato genome (*S. lycopersicum* L.) were that the markers should be different loci, and the repeated region should contain three or four nucleotides, which facilitates their visualization on an agarose gel. The selected markers were located at a distance of 6.0 cM to 146.0 cM from each other within the Tomato- EXPEN 2000 genomic map of chromosomes. This study included 19 SSR primers used for molecular characterization, selected from the Sol Genomics Network (https://solgenomics.net/) database, presented in Table 2.

Molecular characterization

DNA was extracted from the young leaves of individual seedlings using the modified CTAB method (Saghai-Maroof et al. 1984) and the Microprep protocol (Fulton et al. 1995). DNA quality and quantity were determined both spectrophotometrically and through 1% agarose gel electrophoresis. SSR analysis was performed at the Nuclear Energy Research Institute, Ankara, Turkey. All PCR amplifications of genomic DNA were carried out in a 50 µL reaction volume containing 50 ng / µL of genomic DNA, 1x PCR buffer, 1 µM of each primer, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$ and 0.5 U of *Taq* DNA polymerase (Sigma, Germany).

Our amplification program included an initial denaturation at 94 °C for 5 minutes; 35 cycles of denaturation for 30 seconds at 94 °C, annealing for 45 seconds at 50-55 °C, and elongation for 45 seconds at 72 °C; plus a final elongation for 10 minutes at 72 °C. Amplification products were separated in 1.5% agarose (Invitrogen) gels in 1x Tris-borate-EDTA (TBE) buffer, stained with ethidium bromide [*]. The comparison of the fragment lengths was done with a GeneRuler 1 kb DNA Ladder (Thermo Fisher Scientific). The bands were visualized under UV light using the Vilber Lourmat Gel Documentation System.

In the 15 studied genotypes, individual SSR alleles were scored as present (1) or absent (0) to generate a binary data matrix, which was analyzed using NTSYS-2.2j software. Genetic similarity estimations were used for the construction of dendrograms using the unweighted pair group method with arithmetic averages (UPGMA; Sokal and Michener, 1958). The cophenetic correlation coefficient was calculated by the Mantel method (Mantel, 1967) to determine the efficiency of clustering. Mantel r values can fall within a range between -1 to 1. An r value of -1 suggests a strong negative correlation, 0 suggests no relationship at all and 1 suggests a strong positive relationship.

Table 1. Fifteen accessions of tomatoes used for the genetic variability study based on nineteen EST-SSR markers.

| S/N | Varieties and mutant lines | Developed in / Maintained by | Origin - Species | Biological status | Code |
|---|---|---|---|---|---|
| 1 | IZK Niki D | VCRI, Plovdiv-Bulgaria | *S. lycopersicum* L. | $F_1$ hybrid | A |
| 2 | Rozovo sartse | VCRI, Plovdiv-Bulgaria | *S. lycopersicum* L. | Variety | B |
| 3 | Aleno sartse | VCRI, Plovdiv-Bulgaria | *S. lycopersicum* L. | Variety | C |
| 4 | Plovdivska karotina | VCRI, Plovdiv-Bulgaria | *S. lycopersicum* L.+ *S. chillense* L | Variety | D |
| 5 | IZK Alya | VCRI, Plovdiv-Bulgaria | *S. pimpinellifolium* L. | Variety | E |
| 6 | Kopnezh | VCRI, Plovdiv-Bulgaria | *S. lycopersicum* L. | $F_1$ hybrid | F |
| 7 | Vodoley | VCRI, Plovdiv-Bulgaria | *S. lycopersicum* L. | $F_1$ hybrid | G |
| 8 | IZK Olimp | VCRI, Plovdiv-Bulgaria | *S. lycopersicum* L. | $F_1$ hybrid | H |
| 9 | Ideal | VCRI, Plovdiv-Bulgaria | *S. lycopersicum* L. | Variety | J |
| 10 | Ayaş | TENMAK, Ankara-Turkey | *S. lycopersicum* L. | Landrace - Initial accession | K |
| 11 | 21 | TENMAK, Ankara-Turkey | *S. lycopersicum* L. | Mutant line | L |
| 12 | 22 | TENMAK, Ankara-Turkey | *S. lycopersicum* L. | Mutant line | M |
| 13 | 50 | TENMAK, Ankara-Turkey | *S. lycopersicum* L. | Mutant line | N |
| 14 | 55 | TENMAK, Ankara-Turkey | *S. lycopersicum* L. | Mutant line | O |
| 15 | 96 | TENMAK, Ankara-Turkey | *S. lycopersicum* L. | Mutant line | P |

Code: presented Fig. 2. UPGMA-based dendrogram

Table 2. SSR primers used for screening fifteen accessions of tomato

| N | Primer names | Chr. | Sequence | Repeat motif | Repeat No. | $T_a$ (°C) |
|---|---|---|---|---|---|---|
| 1 | SSR19 | 9 | CCGTTACCTTGGTCCATCAC (F)<br>GGGAGATGCCACATCACATA (R) | AT | 16 | 53 |
| 2 | SSR22 | 2 | GATCGGCAGTAGGTGCTCTC (F)<br>CAAGAAACACCCATATCCGC (R) | AT | 11 | 53 |
| 3 | SSR38 | 8 | GTTTCTATAGCTGAAACTCAACCTG (F)<br>GGGTTCATCAAATCTACCATCA (R) | TCT | 8 | 50 |
| 4 | SSR40 | 2 | TGCAGGTATGTCTCACACCA (F)<br>TTGCAAGAACACCTCCCTTT (R) | AC,<br>GC | 7<br>7 | 52 |
| 5 | SSR51 | 1 | CTACCCTGGTCTTGGTGGAA (F)<br>AAAGGATGCTCTAGCTTCTCCA (R) | ACAA | 6 | 50 |
| 6 | SSR61 | 1 | ATGCCTTATGGAAACAACGC (F)<br>CGGGTGTACGAATGTCTTTG (R) | GA | 24 | 50 |
| 7 | SSR66 | 2 | TGCAACAACTGGATAGGTCG (F)<br>TGGATGAAACGGATGTTGAA (R) | ATA | 8 | 50 |
| 8 | SSR76 | 11 | ACGGGTCGTCTTTGAAACAA (F)<br>CCACCGGATTCTTCTTCGTA (R) | CGG | 7 | 50 |
| 9 | SSR80 | 11 | GGCAAATGTCAAAGGATTGG (F)<br>AGGGTCATGTTCTTGATTGTCA (R) | TTTCA<br>GTACAA<br>CAA | 2<br>2<br>7 | 50 |
| 10 | SSR96 | 2 | GGGTTATCAATGATGCAATGG (F)<br>CCTTTATGTCAGCCGGTGTT (R) | AT | 12 | 55 |
| 11 | SSR124 | 12 | TCAATCCATCACACCTTGGA (F)<br>GAGGAAGAAGACCACGCAAA (R) | CACC,GA | 2,7 | 50 |
| 12 | SSR150 | 1 | ATGCCTCGCTACCTCCTCTT (F)<br>AATCGTTCGTTCACAAACCC (R) | CTT | 7 | 50 |
| 13 | SSR156 | 1 | CACGCCTATGCACCTTTCTT (F)<br>CTTCAAGGCTAAACCTCCGA (R) | TCT | 7 | 50 |
| 14 | SSR223 | 10 | TGGCTGCCTCTTCTCTGTTT (F)<br>TTTCTTGAAGGGTCTTTCCC (R) | TCT | 7 | 55 |
| 15 | SSR231 | 3 | TGCCAATCCACTCAGACAAA (F)<br>TGGATTCACCAAGGCTTCTT (R) | TA | 10 | 50 |
| 16 | SSR270 | 1 | AGCTCAAGGCTTCTGTTGGA (F)<br>AACCACCTCAGGCACTTCAT (R) | GAA,<br>GGAGAA | 5,7 | 50 |
| 17 | SSR356 | 2 | ACCATCGAGGCTGCATAAAG (F)<br>AACCATCCACTGCCTCAATC (R) | AT | 20 | 55 |
| 18 | SSR586 | 2 | TCCATCTAAGGTCTTTGCCG (F)<br>ACAAAGGAAGTGGGAGAGCA (R) | AAC | 6 | 55 |
| 19 | SSR590 | 5 | TCTCAAAGTCGTTCCTTCTTGA (F)<br>GGAAGAGAAACGCGGACATA (R) | TC,AC | 6,4 | 55 |

F = forward primer; R = reverse primer

## Results

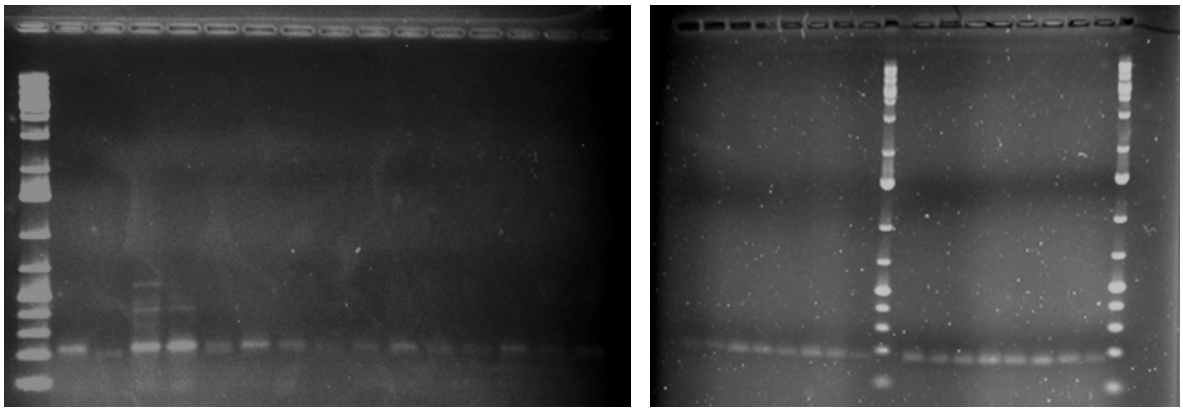
All microsatellite markers selected in the present study were highly reproducible; 15 generated polymorphic profiles (Fig. 1a), and the other four SSR markers (SSR66, SSR156, SSR231, SSR590) led to amplification of the monomorphic profile (Fig. 1b). The number of amplified fragments for all varieties, $F_1$ hybrids and mutant lines for each of the studied markers, and the number of generated profiles, are summarized in Table 3.

SSR19 generated five profiles. The first tomato profile included IZK Niki $F_1$, Rozovo sartse, IZK Olimp $F_1$, initial, No. 50, and No. 96, amplifying three fragments: 250 bp, 1300 bp, and 1800 bp. The second profile included Aleno sartse and Plovdivska karotina (250 bp, 400 bp, 1300 bp, 1800 bp). The genotypes IZK Alya, Kopnezh $F_1$ and Vodoley $F_1$ generated a third profile - only one fragment (250 bp) was amplified. A fourth profile was generated by the genotypes Ideal, No. 21, and No. 55 (1300 bp, 1800 bp), and a fifth by a mutant line No. 22 (250 bp, 1300 bp, 1400 bp, 1800 bp).

SSR22, located on the second chromosome of tomatoes,generated five profiles. The first profile includes four genotypes: IZK Niki $F_1$, Rozovo sartse, Plovdivska karotina and No. 22, amplifying two fragments, 1600 bp and 1650 bp. The second profile includes 4 genotypes: Aleno sartse, IZK Alya, IZK Olimp $F_1$, and No. 21, in which a fragment with a length of 1650 bp is amplified. Vodoley $F_1$ and No. 96 generated a third profile with two fragments amplified, 300 bp and 1600 bp. In the initial landrace (Ayaş), No. 50, and No. 55, three fragments (300 bp and 1600 bp, 1650 bp) are amplified, giving the fourth profile. The fifth profile is characterized by missing fragments.

SSR38 generated five profiles. The first included the varieties IZK Niki $F_1$, IZK Alya, IZK Olimp $F_1$ and Ideal, amplifying a fragment with a length of 600 bp. The Rozovo sartse genotype amplified two fragments, 500 bp and 600 bp. The third profile is generated by Aleno sartse, Kopnezh $F_1$ and Vodoley $F_1$ (250 bp, 500 bp, 600 bp). Plovdivska karotina had five fragments (250 bp, 500 bp, 600 bp, 1050 bp, 1600 bp). The fifth profile included mutant lines and their initial landrace (Ayaş), with an amplified fragment of 250 bp.

SSR40 generated six polymorphic profiles. Three of the profiles included two genotypes: Rozovo sartse and Vodoley



(a) (b)

Figure 1. Example of (1a) polymorphic and (1b) monomorphic profile of SSR-PCR products amplified with SSR76 (P) and SSR590 (M). Lane identification refers to the genotypes as listed in Materials and Methods; GeneRuler 1 kb DNA Ladder was used. (1 - IZK Niki D $F_1$; 2 – Rozovo sartse; 3 - Aleno sartse; 4 – Plovdivska karotina; 5 – IZK Alya; 6 – Kopnezh $F_1$; 7 – Vodoley $F_1$; 8 – Olimp $F_1$; 9 – Ideal; 10 – Ayaş; 11 - No.21; 12 - No.22; 13 - No.50; 14 - No.55; 15 - No.96)

Table 3. Number of bands and profiles generated using SSR markers.

| SSR marker | Total number of different bands | Number of profiles generated |
|---|---|---|
| SSR19 | 4 | 5 |
| SSR22 | 3 | 5 |
| SSR38 | 5 | 5 |
| SSR40 | 4 | 6 |
| SSR51 | 7 | 8 |
| SSR61 | 4 | 4 |
| SSR66 | 1 | 1 |
| SSR76 | 4 | 5 |
| SSR80 | 2 | 3 |
| SSR96 | 2 | 3 |
| SSR124 | 5 | 3 |
| SSR150 | 3 | 4 |
| SSR156 | 1 | 1 |
| SSR223 | 2 | 2 |
| SSR231 | 1 | 1 |
| SSR270 | 3 | 2 |
| SSR356 | 8 | 9 |
| SSR586 | 2 | 2 |
| SSR590 | 1 | 1 |
| Average | 3,26 | 3,68 |

$F_1$ (300 bp); Kopnezh $F_1$ and No. 21 (400 bp, 800 bp, 1400 bp); and No. 22 and No. 50 (400 bp, 800 bp, 1400 bp, 1800 bp). No. 55 generates the fourth profile (400 bp, 1400 bp). The fifth profile included four Bulgarian varieties (IZK Niki $F_1$, Aleno sartse, Plovdivska karotina, IZK Alya) and one mutant line, represented by a 400 bp fragment. The sixth profile is characterized by missing fragments.

SSR51 generated seven profiles in the studied representatives. The first profile (250 bp) included IZK Niki $F_1$, Rozovo sartse, and Aleno sartse. Five of the profiles were generated by single representatives: Plovdivska karotina (400 bp, 500 bp, 1200 bp), IZK Alya (400 bp, 1200 bp, 1700 bp, 1800 bp, 1900 bp), Kopnezh $F_1$ (400 bp, 750 bp, 1200 bp, 1700 bp, 1800 bp, 1900 bp), Vodoley $F_1$ (250 bp, 400 bp, 750 bp), and IZK Olimp $F_1$ (400 bp). Ideal and initial landrace (Ayaş) gave a seventh profile (250 bp, 400 bp).

SSR61 generated four profiles. The first profile amplified fragments 400 bp, 550 bp, and 1100 bp in IZK Niki $F_1$. Second profile was generated from Kopnezh $F_1$ (300 bp, 550 bp). The genotypes Vodoley $F_1$ and IZK Olimp $F_1$ had three fragments – 300 bp, 550 bp, 1100 bp. The fourth profile generated for Rozovo sartse, Aleno sartse, Plovdivska karotina, IZK Alya and Ideal had an amplified fragment of 550 bp.

SSR76 generated five individual profiles: Rozovo sartse (550 bp), Aleno sartse (600 bp, 1100 bp, 1300 bp), Plovdivska karotina (600 bp, 1100 bp), IZK Alya (550 bp, 600 bp), and one profile (600 bp) from the other studied Bulgarian genotypes and Turkish mutant lines.

SSR96, located on the second chromosome, generated three profiles. The first profile was amplified in three varieties (IZK Niki $F_1$, IZK Olimp $F_1$ and Vodoley $F_1$), characterized by a 2500 bp fragment. The second profile included Aleno sartse, Plovdivska karotina, Kopnezh $F_1$, Vodoley $F_1$, and IZK Olimp $F_1$, which had two fragments (600 bp, 2500 bp). In genotypes Rozovo sartse and IZK Alya, no fragments were generated, which was reported in this study as an absence of amplified alleles.

SSR124 generated three profiles. The first profile included IZK Niki $F_1$, Rozovo sartse, Aleno sartse, IZK Alya, Ideal, and mutant lines No. 21 and No. 22, amplifying a 250 bp fragment. The second profile was generated from Plovdivska karotina and Kopnezh $F_1$ (1200 bp, 1500 bp, 2100 bp, 2200 bp, 2300 bp). The third profile was generated from the Bulgarian hybrids Vodoley $F_1$ and IZK Olimp $F_1$, and Turkish mutant lines No. 50, No. 55, No. 96 and their initial (250 bp, 1200 bp, 1500 bp, 2100 bp, 2200 bp, 2300 bp).

SSR150, located on the first chromosome, generated four profiles. The first profile was present in all studied representatives (600 bp), and additional fragments were amplified in the following varieties, constructing individual profiles for Rozovo sartse (250 bp), Plovdivska karotina (600 bp, 1100 bp), and IZK Alya (250 bp, 600 bp).

SSR223 generated two profiles. A 450 bp fragment is amplified in all genotypes studied. In Kopnezh $F_1$, an additional fragment of 2300 bp was amplified.

SSR270 generated two profiles. The first profile includes Bulgarian tomato varieties IZK Niki $F_1$, Rozovo sartse, Aleno surtse, Plovdivska karotina, and IZK Alya, amplifying fragments with lengths of 500 bp and 700 bp. The second profile was generated by four Bulgarian cultivars, five Turkish mu-

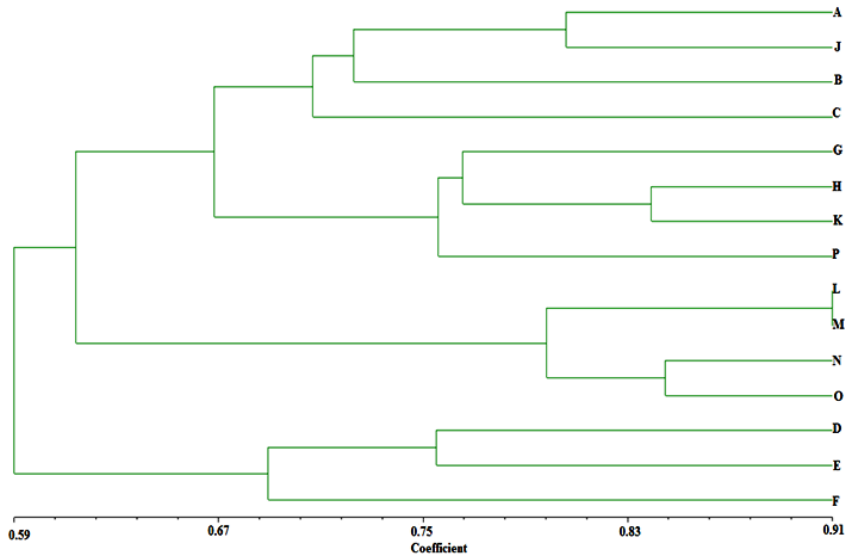



Figure 2. UPGMA-based dendrogram showing genetic similarities among 15 tomato genotype representatives. Codes shown in Table 1.

tant lines ($M_7$), and their initial, in which an additional 1300 bp fragment was amplified.

SSR356 generated nine profiles. Genotypes of No. 50, Vodoley $F_1$, Ideal, and IZK Niki $F_1$ built individual profiles with amplified fragment lengths, respectively, of 600, 700, 1550, and 1800; 750 and 1550; 1550 and 1800; and 1550, 1700, and 1800. Genotypes for No. 55 and Plovdivska karotina included one fragment (600 bp), as did No. 21 and No. 22 (600 bp and 700 bp, respectively). The seventh profile was generated for genotypes Aleno sartse, IZK Alya, Kopnezh $F_1$, IZK Olimp $F_1$, initial, and No. 96 (500 bp, 750 bp, 1550 bp, 1800 bp). Genotypes also had the following amplified fragments in common: 500 bp, 750 bp, 1550 bp, and 1800 bp.

SSR586, located in the second chromosome, generated two profiles. The first is included in all studied genotypes, a product with a length of 1800 bp. The second profile was generated from the Aleno sartse genotype, with a second amplified fragment of 1100 bp.

In this study, the genetic fingerprinting of 15 tomato genotypes (5 varieties, 4 $F_1$ hybrids, 5 mutant lines and their parent) was performed using 19 SSR primers. A total of 62 bands were amplified across the lines, revealing an average of 3.26 bands per primer and 3.68 profiles per marker (Table 3). The scoring and analysis of the bands resulted in the construction of a UPGMA-based dendrogram showing the genetic similarities of genotypes under study, calculated using Nei and Li's coefficient (Fig. 2).

The dendrogram structure was justified on a distance coefficient and a clustering method previously described by Kim et al. (1993).

Analysis of the results obtained from genetic distances and UPGMA - based dendrogram (Fig. 2) revealed that all 15 genotypes were grouped into two major groups. The first cluster included 12 accessions distributed in three subgroups. The first included 4 varieties: A (IZK Niki $F_1$), J (Ideal), B (Rozovo sartse), and C (Aleno sartse). Genetically, A and J were the closest, B was centered, and C is linked. The second subgroup included two Bulgarian varieties, G (Vodoley $F_1$) and H (IZK Olimp $F_1$), and one Turkish mutant line P (No. 96) and its initial (Ayaş) landrace. IZK Olimp $F_1$ and the initial (Ayaş) were grouped into one subgroup, and Vodoley $F_1$ and No. 96 surrounded them. This result gave reason to believe that a genetic difference between the two representatives can be reported. The third subgroup includes 4 varieties distributed between two subgroups. Both subgroups included two variants each: L and M (mutant lines 21 and 22), and N and O (mutant lines 50 and 55). The second major cluster included three varieties: D (Plovdivska karotina), E (IZK Alya), and F (Kopnezh $F_1$).

From the Mantel test (Mantel 1967) conducted based on a regression analysis, in which the variables are distance dissimilarity (or similarity) matrices summarizing all pairwise sample combinations, results for the spatial distribution results show 105 points for identification, with the matrix correlation: r = 0.75469 (Fig. 3). The calculated matrix correlation value is close to 1 and suggests a strong positive relationship and shows distance dissimilarity.

## Discussion

The results obtained in the present study show that all varieties, hybrids, and lines belonging to the species *S. lycopersicum* L. are grouped in one main cluster with four subclusters, except for the $F_1$ hybrid Kopnezh, which is grouped in the second main cluster with two other varieties whose origin is related to wild species. The latter are Plovdivska karotina, obtained by interspecific hybridization between the cultivated species *S. lycopersicum* L. and the wild species *S. chillense* L., and IZK Alya, which belongs to the wild species *S. pimpinellifolium* L. The last two varieties are grouped in the same subcluster. They also differ from the other studied varieties in their morphological features.

Through the use of molecular markers, a cluster analysis confirming the clear differentiation of wild species from cultivated ones was performed. The two genotypes from the wild species were also analyzed with SSR markers by Todorovska et al. (2014), who demonstrated that IZK Alya is the most diverse variety, forming its own cluster in the UPGMA dendrogram.

Based on the statistical analysis of the results obtained the most closely related mutant to the SSR profiles of the initial

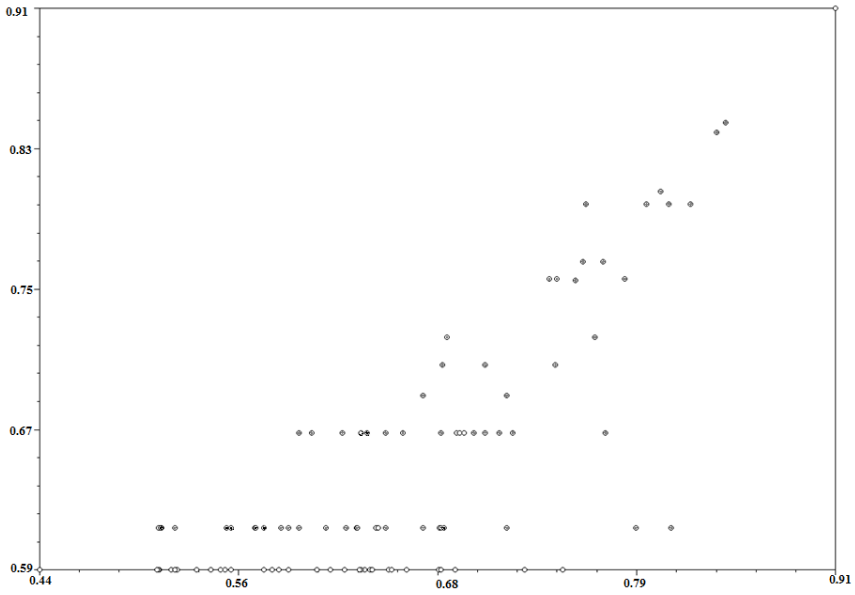



Figure 3. Mantel method used with tomato varieties, $F_1$ hybrids and mutant lines analyzed with nineteen microsatellite markers. Matrix correlation: r = 0.75469.

genotype is No. 96 compared to all the rest 4 studied mutants. The genetic similarity between mutant lines No. 21 and No. 22, and the mutant lines No. 50 and No. 55 is also demonstrated.

The data on the amplified monomorphic profile using SSR 66 with an 8-repeat trinucleotide motif (ATA) from the genotypes in this study correlated with the results of Gharsallah et al. (2016), who reported a relatively low value of polymorphism with its primer. Their results for the other compatible tested markers (SSR 19, SSR 22) also coincide with our results. Benor et al. (2008) reported an average of 4.3 alleles per locus after testing 38 SSR loci on seven inbred lines of tomatoes.

In a study by Gharsallah et al. (2016), out of 25 analyzed SSR genomic markers, 19 were polymorphic, and 70 alleles were detected across the twenty genotypes screened, with an average of 3.68 alleles per locus. In our investigation, out of 19 SSR genomic markers, 15 were polymorphic, which allowed a total of 62 alleles to be detected across the screened genotypes, with an average of 3.26 alleles per locus.

The results obtained here correlated with data reported by Zhou et al. (2015b) involving 15 polymorphic genomic SSRs in 50 studied tomato genotypes. Sixty-four alleles were detected by Gharsallah et al. (2016), with a mean of 4 alleles per primer.

The set of varieties included in this study were characterized by low polymorphism, because sufficient polymorphic markers were often missing between closely related tomato genotypes and within varieties of the same species. Most molecular markers were developed on the basis of polymorphisms between the cultivated tomato and their wild forms. The conducted study, however, gives reason to believe that the combination of microsatellites is useful for studying and establishing genetic diversity among tomato varieties that are closely related to each other. The microsatellite markers studied in the present study were first investigated by Frary et al. (2005). SSR increased the efficiency and accuracy of population genetics analysis based on these markers compared with other markers (Foolad 2007, Wang et al. 2009, Duca et al. 2013, Umar et al. 2018). The combination of high reproducibility of fragments and established polymorphism confirms the possibility of applying the technique in identifying varieties for copyright protection, as well as in hybridization programs.

## Conclusion

The SSR-based microsatellite method has been introduced for tomato genotyping. The molecular genotyping enabled to identify different SSR profiles of the studied Bulgarian varieties and $F_1$ hybrids, and Turkish mutant lines among them and with their initial landrace.

The results achieved have a practical application in the selection of tomatoes. They can be used to develop hybrids, assess the level of genetic diversity in the collection, and conduct a variety of theoretical research studies. The obtained data confirm the efficacy of SSR markers as highly variable markers, detecting the single codominant locus applicable for the differentiation of genetically related varieties. The findings show that for future studies, the use of more and different types of markers would enable studies using more detailed data to understand the genetic differences of breeding materials.

**Acknowledgement.** This research was funded by International Atomic Energy Agency, grant number BUL/5/016 and RER/5/024.

**References**

Alenazi, M.M., Shafiq, M., Alsadon, A.A. (2020): Improved functional and nutritional properties of tomato fruit during cold storage. Saudi Journal of Biological Sciences 27(6): 1467-1474.

Alzahib, R.H., Migdadi,H.M., Ghamdi, A.A., Alwahibi, M.S., Afzal, M., Elharty, E.H., Alghamdi, S.S. (2021): Exploring genetic variability among and within Hail tomato landraces based on sequence-related amplified polymorphism markers. Diversity 13(135): 1-12.

Anonymous (2020): TÜRKTOB (Türkiye Tohumcular Birliği) Tohumculuk Sektör Raporu. <https://turktob.org.tr/upload/2020_TOHUMCULUK_SEKTOR_RAPORU-.pdf>

Areshchenkova, T., Ganal, M.W. (2002): Comparative analysis of polymorphism and chromosomal location of tomato microsatellite markers isolated from different sources. Theoretical Applied Genetics 2(3): 229-235.

Benor, S., Zhang, M., Wang, Zh., Zhang, H. (2008): Assessment of genetic variation in tomato (*Solanum lycopersicum* L.) inbred lines using SSR molecular markers. Journal of Genetics and Genomics 35: 373-379.

Bernatzky, R., Tanksley, S.D. (1986): Toward a saturated linkage map in tomato based on isozymes and random complementary DNA sequences. Genetics 112: 887-898.

Duca, M., Port, A., Şestacova, T., Siniauskaya, M., Aksyonova, E., Davydenko, O. (2013): Microsatellite marker application in sunflower (*Helianthus annuus* L.) fingerprinting. Biotechnology & Biotechnological Equipment 27(3): 3772-377.

El-Mansy, A.B., El-Moneim, D.A., Alshamrani, S.M., Alsafhi, F.A., Abdein, M.A., Ibrahim, A.A. (2021): Genetic diversity analysis of tomato (*Solanum lycopersicum* L.) with morphological, cytological and molecular markers under heat stress. Horticulturae 7(65): 1-18.

Foolad, M.R. (2007): Genome mapping and molecular breeding of tomato. International Journal of Plant Genomics 2007: 64358.

Frary, A., Eck, J.V. (2005): Organogenesis from transformed tomato explants. Methods in Molecular Biology 286: 141-150.

Frary, A., Xu, Y., Liu, J., Mitchell, Sh., Tedeschi, E., Tanksley, S. (2005): Development of a set of PCR-based anchor markers encompassing the tomato genome and evaluation of their usefulness for genetics and breeding experiments. Theoretical Applied Genetics 111: 291-312.

Fulton, T.M., Chunwongse, J., Tanksley, S.D. (1995): Microprep protocol for extraction of DNA from tomato and other herbaceous plants. Plant Molecular Biology Report 13: 207-209.

Ganeva, D., Aleksandrova, K., Pevicharova, G., Masheva, S., Bogatzevska, N. (2016): Increasing the level of resistance towards bacterial spot and speck in tomato cultivars with high biological value fruits. 2nd International Symposium for Agriculture and Food. Section 5, Vegetable, flower and decorative plants production: 403-411.

Gharsallah, C., Fakhfakh, H., Grubb, D., Gorsane, F. (2016): Effect of salt stress on ion concentration, proline content, antioxidant enzyme activities and gene expression in tomato cultivars. AoB PLANTS 8: plw055.

He, G., Meng, R., Newman, M., Gao, G., Pittman, R.N., Prakash, C.S. (2003): Microsatellites as DNA markers in cultivated peanut (*Arachis hypogaea* L.). BMC Plant Biology 3(3): 1-6.

Kantoğlu, K.Y., Tepe, A., Kunter, B., Fırat, A.F., Peşkircioğlu, H. (2014): Vegetable crops breeding by induced mutation and a practical case study of *Capsicum annuum* L. pp.41-56. In: Tomlekova, N.B., Kozgar, M.I., Wani, M.R. (eds), Mutagenesis: exploring genetic diversity of crops. Wageningen.

Kim, J.L., Nikolov, D.B., Burley, S.K. (1993): Co-crystal structure of TBP recognizing the minor groove of a TATA element. Nature 365(6446): 520-527.

Kulus, D. (2018): Tomato molecular breeding – a mini review of latest achievements. Nauka, Przyroda Technologie 12(1): 65-72.

Mantel, N. (1967): The detection of disease clustering and a generalized regression approach. Cancer Research 27: 209-220.

Naika, Sh., Lidt de Jeude, J., Goffau, M., Hilmi, M., Dam, B. (2005): Cultivation of tomato – production, processing and marketing. Agromisa Foundation and CTA, Wageningen, Agrodok 17: 1-92.

Nei, M., Li, W.H. (1979): Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences of the United States of America 76(10): 5269-5273.

Rick, C., Simmonds, N. (1976): Tomato *Lycopersicon esculentum* (Solanaceae). Evolution Crop Plants: 268-273.

Smulders, M., Bredemeijer, G., Rus- Kortekaas, W., Arens, P., Vosman, B. (1997): Use of short microsatellites from database sequences to generate polymorphisms among *Lycopersicon esculentum* cultivars and accessions of other Lycopersicon species. Theoretical Applied Genetics 94: 264-272.

Shirasawa, K., Isobe, S., Hirakawa, H., Asamizu, E., Fukuoka, H., Just, D., Rothan, CH., Sasamoto, S.H., Fujishiro, Ts., Kishida, Y., Kohara, M., Tsuruoka, H., Wada, TS., Nakamura, Y., Sato, SH., Tabata, S. (2010): SNP discovery and linkage map construction in cultivated tomato. DNA Research 17: 381-391.

Sokal, R.R., Michener, C.D. (1958): A statistical method for evaluating relationships. University of Kansas Science Bulletin 38: 1409-1448.

Tanksley, S.D., Ganal, M.W., Prince, J.P., Vicente, M.C., Bonierbale, M.W., Broun, P., Fulton, T.M., Giovannoni, J.J., Grandillo, S., Martin, G.B., Messeguer, R., Miller, J.C., Paterson, A.H., Pineda, O., Roder, M.S., Wing, R.A., Wu, W., Young, N.D. (1992): High-density molecular linkage maps of the tomato and potato genomes. Genetics 132: 1141-1160.

Tilahun, M., Angassa, A., Abebe, A. (2017): Community-based knowledge towards rangeland condition, climate change, and adaptation strategies: the case of Afar pastoralists. Ecological Processes 6(29): 1-13.

Todorovska, E., Ivanova, A., Ganeva, D., Pevicharova, G., Molle, E., Bojinov, B., Radkova, M., Danailov, Z. (2014): Assessment of genetic variation in Bulgarian tomato (*Solanum lycopersicum* L.) genotypes, using fluorescent SSR genotyping platform. Biotechnology & Biotechnological Equipment 28(1): 68-76.

Umar, J., Aliyu, A.A., Kasimu, S., Lawal, A. (2018): Identification and analysis of salt responsive candidate gene based on SSRs from tomato (*Solanum lycopersicum* L.). International Journal of Cell Science & Molecular Biology 5(1): 555-653.

Wang, Q., Sang, X., Ling, Y., Zhao, F., Yang, Z., Li, Y., He, G. (2009): Genetic analysis and molecular mapping of a novel gene for zebra mutation in rice (*Oryza sativa* L.). Journal of Genetics and Genomics 36: 679-684.

Zamir, D. (2001): Improving plant breeding with exotic genetic libraries. Nature Reviews Genetics 2(12): 983-989.

Zorb, Ch., Piepho, H.P., Zikeli, S., Horneburg, B. (2020): Heritability and variability of quality parameters of tomatoes in outdoor. Research 2020: art.6707529.

Zhou, R., Wu, Z., Cao, X., Jiang, F.L. (2015): Genetic diversity of cultivated and wild tomatoes revealed by morphological traits and SSR markers. Genetics and Molecular Research 14(4): 13868-13879.