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# Molecular Evaluation of Genetic Variability in Tomato (*Lycopersicon esculentum* Mill.) Genotypes by Microsatellite Markers

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**Summary:** The objective of this research was to assess genetic diversity using eight microsatellite markers in 30 tomato genotypes from the collection of the Institute of Field and Vegetable Crops in Novi Sad. The SSR markers were selected from publicly available data and *Solanaceae Genome Network* database. Genotypes were grouped into three clusters, using Ward`s hierarchical clustering method and Euclidean distance measure. Markers SSR248, TMS9, TMS42 and SSR111 had very high PIC (Polymorphism information content) values and can be recommended for the future studies.
**Key words:** genetic variability, genetics, *Lycopersicon esculentum*, microsatellites, SSR markers, tomatoes

## Introduction

Harvested areas, quantity production and human consumption provided an important place for tomato (*Lycopersicon esculentum* Mill.) among other vegetable plants. According to FAO statistical data (Food and Agriculture Organization of the United Nations 2011), the global harvested area of tomato was 4,751,530 ha in 2011. Although derived from highly variable natural habitat, a huge part of genetic variability was lost due to inbreeding during tomato domestication and intensive artificial selection. A huge number of tomato varieties and hybrids were developed by tomato breeders with the aim to overcome existing genotypes in terms of yield, resistance to biotic and abiotic stress and fruit quality. Access to divergent breeding material is necessary for achieving these objectives. Despite the highly diverse natural habitat of the genus *Lycopersicon* that resulted in great variability of its species, cultivated tomato has a very narrow genetic base. Assessment of genetic diversity in any crop species provides a basis for devising future strategies for crop improvement, conservation and sustainable use (Yi et al. 2008). Molecular markers represent useful tool in these types of studies. Simple sequence repeats (SSRs), also known as microsatellites, have very important role in molecular research having high reproducibility, multi-allelic nature, co-dominant inheritance, high abundance and wide genome coverage (El-Awady et al. 2012). Microsatellites are widely used in studies in different plant species (Stich et al. 2006, Cadalen et al. 2010, Brbaklić et al. 2010, Trkulja et al. 2011, Shah et al. 2013). Numerous studies have pointed to the efficacy of SSR markers for determination of genetic diversity in the genus *Solanum* (Alvarez et al. 2001, He, Poysa, & Yu 2003, Frary et al. 2005, Garcia-Martinez et al. 2006, Hu et al. 2012). As a consequence of current breeding strategies, diverse local populations and old cultivars are suppressed by modern, high yielding cultivars, uniform with a narrow allelic variability at agronomically important loci. Since the loss of specific alleles means loss of opportunities to develop new and improve existing varieties and

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hybrids, the aim of this research was to evaluate genetic variability of 30 tomato genotypes using eight SSR markers.

## Materials and Methods

Thirty genotypes from tomato collection of the Institute of Field and Vegetable Crops (Novi Sad, Serbia) were chosen in order to assess genetic diversity using eight SSR markers (Tab. 1). Plant material originating from different parts of the world included 4 wild *Lycopersicon* species, 4 local populations, 16 old cultivars, 5 commercial cultivars and 1 breeding line. From the Sol Genomics Network (SGN; available at http://solgenomics.net/), after Bombarely et al. (2011) and publicly available data (Frary et al. 2005, Chen et al. 2009) the following microsatellite markers were selected: SSR248, SSR111, SSR9, SSR66, SSR304, SSR80, TMS9 and TMS42 (Tab. 2). The seedlings were grown in wooden boxes in greenhouse for two weeks. Genomic DNA was extracted from leaf tissue using modified CTAB isolation method (Doyle

Table 1. Analyzed tomato genotypes from the collection of the Institute of Field and Vegetable Crops, Novi Sad

| Collection number | Common genotype name | Origin | Type of material |
|---|---|---|---|
| S 3 | Alice Roosevelt | USA | old cultivar |
| S 15 | Novosadski niski | Serbia | commercial cultivar |
| S 30 | Idyll | Germany | old cultivar |
| S 42 | Kurtovski | Bulgaria | old cultivar |
| S 46 | Bačka | Serbia | commercial cultivar |
| S 50 | Knjaz | Serbia | commercial cultivar |
| S 70 | Skopski rani | FYROM | local population |
| S 99 | Novosadski rani | Serbia | local population |
| S 100 | Novosadski export | Serbia | local population |
| S 112 | Cverglan | Hungary | local population |
| S 119 | *Licopersicon peruvianum* | South America | wild species |
| S 120 | *Licopersicon pimpinelliofolium* | South America | wild species |
| S 122 | Pegaz | Serbia | commercial cultivar |
| S 200 | Acumare le caste | Italy | old cultivar |
| S 212 a | Micado wioleto | Italy | old cultivar |
| S 214 | *Lycopersicon hirsutum f. glabratum* | Western parts of South America (Galapagos) | wild species |
| S 218 | *Lycopersicon esculentum var. cerasiforme* | Western parts of South America | wild species |
| S 319 | Gloria di Milano | Italy | old cultivar |
| S 320 | Sunny Brok | USA | old cultivar |
| S 332 | Antimold B | USA | old cultivar |
| S 336 | San Marzano | Italy | old cultivar |
| S 370 | Lampadina 7 | Italy | old cultivar |
| S 399 | VF 145-21-4 | Denmark | breeding line |
| S 427 | Hode | Netherlands | old cultivar |
| S 477 | Indiana | USA | old cultivar |
| S 534 | NR 189 | Denmark | old cultivar |
| S 545 | Benarys Gartenfreude | Germany | old cultivar |
| S 563 | Florida MH-1 | USA | old cultivar |
| S 564 | Ontario red | Canada | old cultivar |
| S 579 | Narvik SPF | Serbia | commercial cultivar |

& Doyle 1990). PCR amplifications were carried out in 10 µl reaction volume: 30 ng genomic DNA, 1×buffer, 2 mM $MgCl_2$, 0.2 mM of dNTPs, 1 unit of Taq polymerase and 10 pmol of reverse and forward primers. PCR products were separated by capillary electrophoresis using the ABI genetic analyzer Prism 3130 (Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA). The amplification products were analyzed by GeneMapper Software Version 4.0. Polymorphism information content (PIC) was calculated according to Anderson, Churchill, Autrque, Tanksley, & Sorrells (1993):

$$PIC = 1 - \sum_{i=1}^{k} pi^2$$

where *pi* is the frequency of the *i*-th allele out of the total number of alleles at an SSR locus, in the set of thirty tomato genotypes, and *k* is a total number of different alleles of a given locus.

Statistical software Statistica 9 (StatSoft Inc. Corporation, Tulsa, USA) was used for genotype clustering using Ward`s hierarchical clustering method and Euclidean distance measure ($d_E$).

**Results and Discussion**

The genotypes were scored for the presence or the absence of the different SSR alleles. Eight loci were detected having total of 31 alleles, resulting in an average allele number of 3.9 per locus. Number of alleles per locus ranged from 3 to 5, while PIC values of selected markers varied from 0.12 (SSR9) to 0.73 (SSR248). Markers SSR248, TMS9, TMS42 and SSR111 were highly informative having PIC values over 0.5. Kwon, Parkl, & Yi (2009) also found a high level of polymorphism for markers SSR248 and SSR111 with PIC values 0.75 and 0.88, respectively. High PIC value for marker TMS9 (0.62) and low value for marker TMS42 (0.23) were reported by He et al. (2003). Genotypes were grouped into three clusters, but it was not possible to distinguish among genotypes with different origins (Fig. 1). The first group consisted of 10 genotypes and included 5 old cultivars, 2 wild species, 2 local populations and 1 commercial cultivar. In this group, no difference was revealed between cultivar S122 from Serbia and old cultivar S319 from Italy. The most distinct genotypes in this group were old cultivar S3 from the USA and old cultivar

Table 2. SSR markers used for molecular evaluation of 30 tomato genotypes

| Marker | Chromo-some | Repeat motif | Primer sequence (5'-3') | No. alleles | PIC |
|---|---|---|---|---|---|
| SSR 248 | 10 | (ta)21 | f: GCATTCGCTGTAGCTCGTTT<br>r: GGGAGCTTCATCATAGTAACG | 5 | 0.73 |
| SSR 111 | 3 | (tc)6<br>(tctg)6 | f: TTCTTCCCTTCCATCAGTTCT<br>r: TTTGCTGCTATACTGCTGACA | 4 | 0.60 |
| SSR 9 | 1 | (ata)10 | f: CCCTTTGCAAGTTCTTCTTCA<br>r: TTCATGAGCCAACATAGGAGG | 3 | 0.12 |
| SSR 66 | 2 | (ata)8 | f: TGCAACAACTGGATAGGTCG<br>r: TGGATGAAACGGATGTTGAA | 3 | 0.24 |
| SSR 304 | 7 | (cca)7 | f: TCCTCCGGTTGTTACTCCAC<br>r: TTAGCACTTCCACCGATTCC | 3 | 0.24 |
| SSR 80 | 11 | (tttcaa)2<br>(gtacaa)2<br>(caa)7 | f: GGCAAATGTCAAAGGATTGG<br>r: AGGGTCATGTTCTTGATTGTCA | 4 | 0.39 |
| TMS 9 | 12 | (gata)26 | f: TTGGTAATTTATGTTCGGGA<br>r: TTGAGCCAATTGATTAATAAGTT | 4 | 0.70 |
| TMS 42 | 11 | (at)17<br>(gt)18 | f: AGAATTTTTTCATGAAATTGTCC<br>r: TATTGCGTTCCACTCCCTCT | 5 | 0.56 |

S564 originating from Canada. Genotypes of the second group included 2 wild species, 1 cultivar in production and 1 old cultivar. The lowest $d_E$ was obtained between cultivar S46 from Serbia and old cultivar S534 originating from Denmark. Wild species *Lycopersicon hirsutum f. glabratum* (S214), originating from the western parts of South America (Galapagos), had the highest $d_E$ from the rest of the second group genotypes, and from the majority of analysed genotypes. The third group, with 14 genotypes, contained 8 old cultivars, 3 commercial cultivars, 2 local populations and 1 breeding line. The lowest $d_E$, in this group was determined between the following genotypes: two Italian, old cultivars, S336 and S370; cultivar S15 (Serbia) and old cultivar S320 (USA); old cultivar S563 (USA) and commercial cultivar S579 (Serbia). Two old cultivars, S336 and S370, except the same origin, have the same elongated „San Marzano" fruit type. Obtained similarity between other genotypes could be the consequence of the small number of loci being used at this stage of study.

No difference was revealed between two old cultivars originating from Germany (S545 and S30), which were grouped separately from the rest of the analysed genotypes.

## Conclusions

Four SSR markers (SSR248, TMS9, TMS42 and SSR111) had very high PIC values and can be recommended for the future studies. Most of the analysed genotypes could be distinguished using eight chosen SSR markers. For better germplasm characterization and development of potential markers for agronomically important traits, more SSR markers should be included in the analysis. This type of research, coupled with the results of field observations, can present a starting point for MAS (Marker Assisted Selection) in tomato breeding.

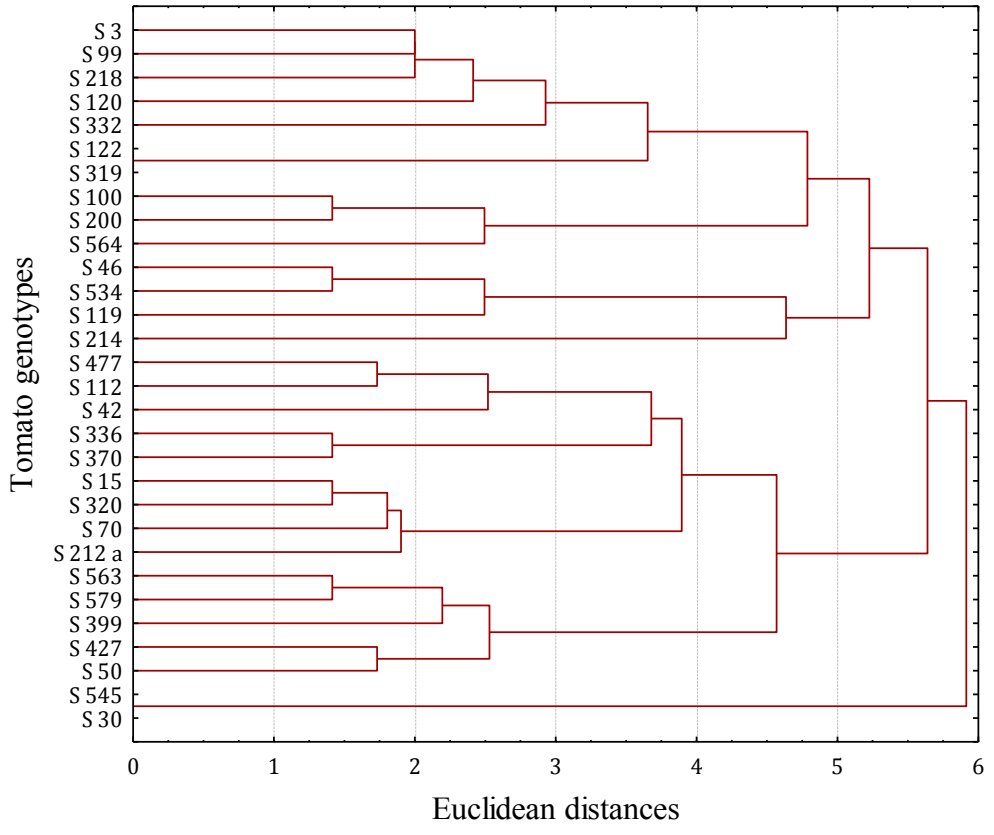


Figure 1. Dendrogram of 30 tomato genotypes based on 8 SSR markers data

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# Molekularna evaluacija genetičke varijabilnosti paradajza (*Lycopersicon esculentum* Mill.) mikrosatelitskim markerima

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**Sažetak:** Paradajz (*Lycopersicon esculentum* Mill.) je jedna od povrtarskih vrsta najviše izučavanih na polju oplemenjivanja, genetike i genomike i jedna od 3000 iz familije *Solanaceae* (pomoćnice). Veličina genoma paradajza iznosi 950 Mbp, a sadrži 77% heterohromatina i 23% euhromatina (Peterson, Price, Johnston i Stack 1996). Iako je diverzitet njegovih prirodnih staništa veoma velik, znatan deo genetičke varijabilnosti paradajza je izgubljen u procesu domestifikacije i intenzivne veštačke selekcije. Cilj ovog istraživanja je procena genetičkog diverziteta 30 genotipova paradajza iz kolekcije Instituta za ratarstvo i povrtarstvo u Novom Sadu korišćenjem 8 mikrosatelitskih markera. SSR markeri su izabrani na osnovu objavljene naučne literature i *Solanaceae Genome Network* baze podataka. Markeri SSR 248, TMS 9, TMS 42 i SSR 111, kod kojih je utvrđena visoka PIC vrednost, mogu se preporučiti za buduća istraživanja.
**Ključne reči:** genetička varijabilnost, genetika, *Lycopersicon esculentum*, mikrosateliti, paradajz, SSR markeri