

Determination of genetic diversity among different tomato varieties using SSR markers

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Abstract: The simple sequence repeats (SSR) have become the preferred molecular markers for variety identification, genetic mapping and phylogenetic analysis. The aim of this study was to evaluate genetic relationships among six morphologically different tomato varieties (var. *grandifolium*, var. *cerasiforme* (red), var. *cerasiforme* (yellow), var. *pruniforme*, var. *pyriforme* and var. *racemigerum*) using nine SSR markers (LECH13, LE21085, LEMDDNa, LEEF1Aa, LELEUZIP, LE20592, TMS9, LE2A11 and LECHSOD). Fragment analysis was conducted using an *Applied Biosystems* DNA analyzer (*ABI 3130*) and *GeneMapper® Software program*. The data were analyzed using *Power Marker Software* and *MEGA3* programs. The results showed that the lowest genetic distance (16.7415) was identified between var. *cerasiforme* (yellow) and var. *cerasiforme* (red), and the largest (34.9859) between var. *pyriforme* and var. *grandifolium*. On the other hand, the

lowest genetic distance (22.1446) was observed between subsp. *subspontaneum* and subsp. *spontaneum*, and the largest (29.7147) between subsp. *subspontaneum* and subsp. *cultum*. Precise dendrograms were created based on the statistical data.

Key words: *Lycopersicon esculentum* Mill.; varieties; SSR; genetic diversity

Introduction

Genetic diversity has to be evaluated in order to establish breeding strategies and to manage genetic resources.

SSR markers have been already efficiently used for studies of genetic diversity, mapping, and variety identification in different crops.

In tomato, many SSR markers have been developed (Smulders *et al.* 1997; He *et al.* 2003), but only a limited number of SSR markers have been mapped (Areshchenkova and Ganai 2002).

Smulders *et al.* (1997), Bredemeijer *et al.* (2002), He *et al.* (2003) confirmed a significant role of SSR markers in survey of genetic diversity and variability of genus *Solanum*, as well as for tomato variety identification.

As the number of varieties continuously increase, the discrimination among cultivars based on morphological traits becomes less and less efficient and molecular markers can be used as a complementary tool. Molecular markers have been successfully applied in registration activities, such as cultivar identification where the goal is to obtain specific pattern for each variety (Lombard *et al.* 2001).

Molecular markers such as SSR markers have more advantages for plant variety identification over the more traditionally used morphological and biochemical markers because of their independence from environmental influences, high level of polymorphism and their almost unlimited availability. These techniques are also likely to be extremely discriminating and much more rapid. SSR markers have the advantages of being multiallelic, highly polymorphic, co-dominant, and assayable by PCR. The first application of SSR markers in plants has been in cultivar identification. A combination of microsatellites can be useful in distinguishing cultivars of tomato, which are genetically very closely related to each other.

The objective of this study was to evaluate genetic relationships among six tomato varieties (*Lycopersicon esculentum* Mill.) using SSR markers.

Material and methods

Plant material: The plant material was obtained from the Gen-bank of Agricultural Institution in Skopje. Six different tomato varieties from three subspecies of *Lycopersicon esculentum* Mill were investigated in this research: var. *grandifolium* from subsp. *cultum* Brezh.; var. *cerasiforme* (with red fruits),

var. *cerasiforme* (with yellow fruits), var. *pruniforme* and var. *pyriforme* from subsp. *subspontaneum* Brezh. and var. *racemigerum* from subsp. *spontaneum* Brezh.

DNA extraction: DNA was extracted from the leaves of tomato grown for a few weeks from 10 individual plants per each variety and from the pooled seeds of each variety. The DNA from leaves was isolated and purified using a Wizard® Genomic DNA purification kit from Promega, while from seeds DNA was isolated using CTAB method (Doyle & Doyle 1987 and Cullings 1992).

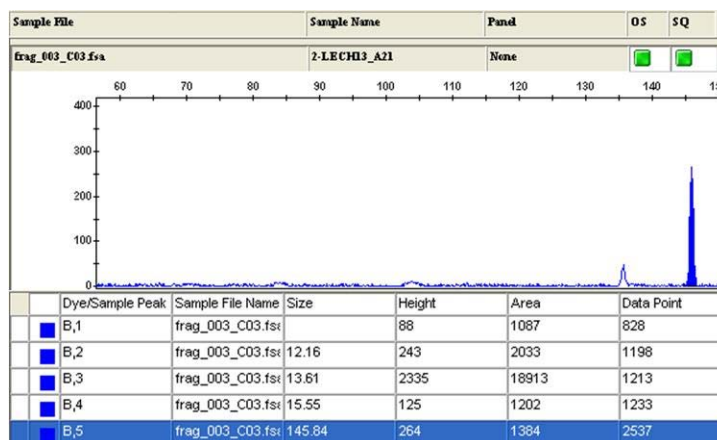
DNA analysis: The standardization of PCR conditions for amplification of 9 polymorphic regions was performed using appropriate primer pairs for the following loci: LECH13, LE21085, LEMDDNa, LEEF1Aa, LELEUZIP, LE20592, TMS9, LE2A11 and LECHSOD. After PCR amplification, the products were analyzed using *Applied Biosystems* DNA analyzer (*ABI 3130*) and *GeneMapper®Software* program. The statistical analyses were carried out using the specific computer programs: POWER MARKER SOFTWARE and MEGA3. The genetic distance estimation was performed according to the method of Slatkin (1995).

Results and discussion

In this survey nine SSR markers were used for determination of genetic diversity among six tomato varieties (*Lycopersicon esculentum* Mill).

After PCR amplification, all 9 SSR markers were able to generate PCR products. Fragment analyses of PCR products were performed on an *Applied Biosystems* DNA analyzer (*ABI 3130*) using *GeneMapper®Software* program. Fragment analysis results were shown as electropherograms of homozygous and heterozygous samples (Figure 1).

a)



b)

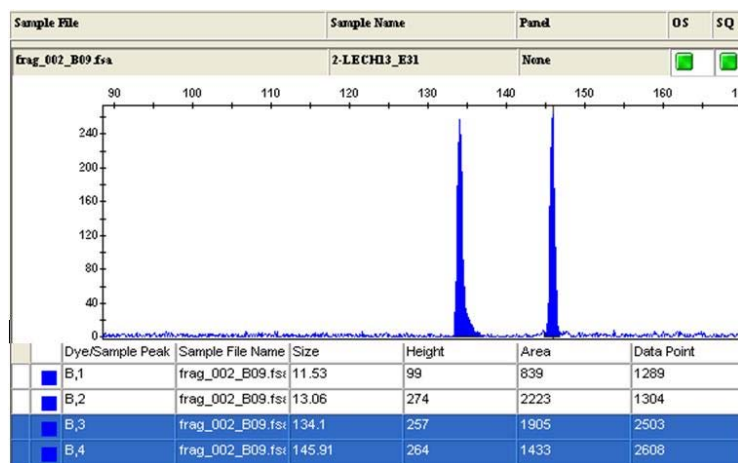


Figure1. Fragment analysis performed on loci LECH13: a) homozygous and b) heterozygous

The results of genetic distance according to the method of Slatkin, in relation to the investigated varieties showed that the lowest genetic distance (16.7415) was estimated between *Lycopersicon esculentum* subsp. *subspontaneum* var. *cerasiforme* (with yellow fruits) and *Lycopersicon esculentum* subsp. *subspontaneum* var. *cerasiforme* (with red fruits), and the largest genetic distance (34.9859) was determined between *Lycopersicon esculentum* subsp. *subspontaneum* var. *pyriforme* and *Lycopersicon esculentum* subsp. *cultum* var. *grandifolium* (Table 1).

Table 1. Results of the genetic distances among the test tomato varieties (according to Slatkin 1995)

	var. <i>cerasiform</i> (red)	var. <i>cerasiform</i> (yellow)	var. <i>grandifolium</i>	var. <i>pruniforme</i>	var. <i>pyriforme</i>	var. <i>racemigeru</i> <i>m</i>
var. <i>cerasiforme</i> (red)	0.0000					
var. <i>cerasiforme</i> (yellow)	16.7415	0.0000				
var. <i>grandifolium</i>	23.4737	27.9057	0.0000			
var. <i>pruniforme</i>	19.8646	20.1435	32.4933	0.0000		
var. <i>pyriforme</i>	22.8229	22.6102	34.9859	24.4848	0.0000	
var. <i>racemigerum</i>	18.1202	20.1979	28.7833	23.8861	26.3743	0.0000

The lowest genetic distance noticed between *Lycopersicon esculentum* subsp. *subspontaneum* var. *cerasiforme* (with yellow fruits) and *Lycopersicon*

esculentum subsp. *subspontaneum* var. *cerasiforme* (with red fruits) is justified and expectable. Namely, according to Brezhnev classification (1964) these varieties belong to the same subspecies (subsp. *subspontaneum*) and they have different fruit color. The relatively similar data for genetic distances among var. *cerasiforme* (with yellow fruits), var. *cerasiforme* (with red fruits), var. *pruniforme* and var. *pyriforme* are justified. According to Brezhnev (1964) these varieties belong to the same subspecies (subsp. *subspontaneum*) and their genetic closeness is completely justified.

Huge genetic distances were noticed between *Lycopersicon esculentum* subsp. *cultum* var. *grandifolium* and the other tomato varieties. These genetic distances are expectable in view of the fact that this variety according to Brezhnev classification (1964) belongs to subsp. *cultum*, as well as the fact that according to Park *et al.* (2004) cultivated tomatoes show limited genetic distance. Similarly, the cluster analyses performed by Noli *et al.* (1999) allowed to clearly distinguish *L. esculentum* accessions from their wild relatives (*L. pimpinellifolium*, *L. racemigerum* and *L. esculentum* var. *cerasiforme*).

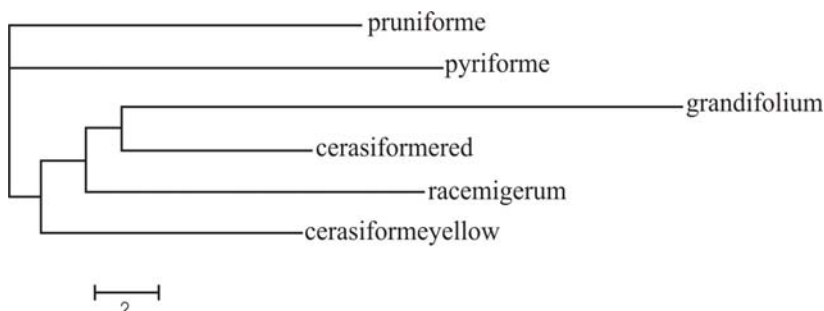


Figure 2. Dendrogram of the tomato varieties (clusterization according to Neighbor-Joining method)

On the other hand, relatively small genetic distance between var. *racemigerum* and representatives of subsp. *subspontaneum* (var. *cerasiforme* - with yellow fruits, var. *cerasiforme* - with red fruits, var. *pruniforme* and var. *pyriforme*) is surprising because according to Brezhnev classification (1964) var. *racemigerum* belongs to subsp. *spontaneum*. Also, the analyses of Kaemmer *et al.* (1995) showed that *L. piminellifolium* (L.) Mill. (syn. *L. racemigerum* Lange according to UPOV Codes since July 1, 2009) are close to *L. esculentum* var. *cerasiforme*. Namely, according to Kaemmer *et al.* (1995) the close association of *L. piminellifolium* and *L. esculentum* var. *cerasiforme* in particular are in good agreement with data derived from chloroplast (Palmer and Zamir 1982) and nuclear RFLPs (Miller and Tanksley 1990) and isozyme analyses (Bretó *et al.* 1993), as well as with the proposed origin in cultivated tomato (Jenkins 1948,

Warnock 1988). These results are in agreement with the results published by Alvarez *et al.* (2001) and Villalta *et al.* (2005).

Based on the genetic distance data among investigated tomato varieties, a precise dendrogram was created (Figure 2). Cluster analyses resulted in three main groups (clusters). The first cluster contains only var. *pruniforme*, and the second includes only var. *pyriforme*. Surprisingly, var. *grandifolium*, var. *cerasiforme* - with red fruits, var. *racemigerum* and var. *cerasiforme* - with yellow fruits belong to the third cluster, although according to Brezhnev (1964) only var. *cerasiforme* - with red fruits and var. *cerasiforme* - with yellow fruits belong to the same subspecies (*subspontaneum*). Probably, the presence of var. *racemigerum* in the third cluster could be associated with Schiemann's theory that this variety is the ancestor of cultivated tomatoes (Aladžajkov 1966). Also, according to Rick (1976) either *L. piminellifolium* may be the direct ancestor of *L. esculentum* or alternatively both could have evolved in parallel from a greed-fruited ancestor. In either case, it is likely that genes from *L. piminellifolium* have played an important part in the evolution of the cultivated tomato (Taylor 1986).

The results of genetic distance determination, according to the method of Slatkin, in relation to investigated subspecies showed that the smallest genetic distance (22.1446) was observed between *Lycopersicon esculentum* subsp. *subspontaneum* and *Lycopersicon esculentum* subsp. *spontaneum*, and the largest (29.7147) between *Lycopersicon esculentum* subsp. *subspontaneum* and *Lycopersicon esculentum* subsp. *cultum* (Table 2).

Table 2. Results on genetic distance among the test tomato subspecies (according to Slatkin 1995)

	subsp. <i>cultum</i>	subsp. <i>spontaneum</i>	subsp. <i>subspontaneum</i>
subsp. <i>cultum</i>	0.0000		
subsp. <i>spontaneum</i>	28.7833	0.0000	
subsp. <i>subspontaneum</i>	29.7147	22.1446	0.0000

The large genetic distances observed between *Lycopersicon esculentum* subsp. *cultum* and *Lycopersicon esculentum* subsp. *subspontaneum*, as well as between *Lycopersicon esculentum* subsp. *cultum* and *Lycopersicon esculentum* subsp. *spontaneum*, are completely justified if we know that according Park *et al.* (2004) cultivated tomatoes show limited genetic distance.

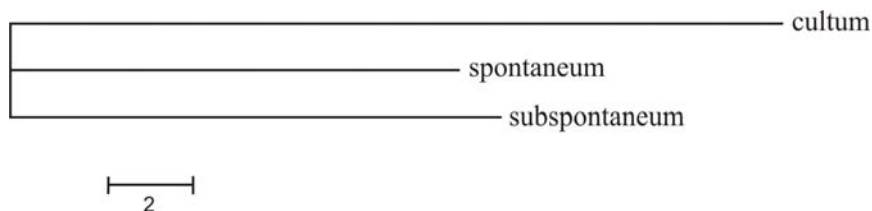


Figure 3. Dendrogram of the tomato subspecies (clusterization according to Neighbor-Joining method)

A precise dendrogram was created based on the data of genetic distance among investigated tomato subspecies (Figure 3). Cluster analyses resulted in three groups (clusters) which is in agreement with the classification of Brezhnev (1964).

Conclusions

This research suggested the following:

The dendrogram based on the data of genetic distance among the test tomato varieties is not completely in accordance with the classification of Brezhnev (1964); as opposed to the dendrogram based on the data of genetic distance among tomato subspecies which is in agreement with the classification of Brezhnev (1964).

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UTVRĐIVANJE GENETIČKE RAZNOLIKOSTI RAZLIČITIH VARIJETETA PARADAJZA PRIMENOM SSR MARKERA

- originalni naučni rad -

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Rezime

Jednostavne ponavljajuće sekvence (*eng.* simple sequence repeats – SSR) kao molekularni markeri prioritetno se primenjuju u identifikaciji varijeteta, genetskom mapiranju i filogenetskoj analizi. Cilj ovog istraživanja bio je određivanje genetskog odnosa između šest morfološki različitih varijeteta paradajza (var. *grandifolium*, var. *cerasiforme* (crveni), var. *cerasiforme* (žuti), var. *pruniforme*, var. *pyriforme* i var. *racemigerum*) primenom devet SSR markera (LECH13, LE21085, LEMDDNa, LEEF1Aa, LELEUZIP, LE20592, TMS9, LE2A11 i LECHSOD). Fragmentarna analiza izvršena je pomoću DNK analizatora *Applied Biosystems (ABI 3130)* i *GeneMapper®* softvera. Dobijeni podaci obrađeni su uz pomoć Power Marker i MEGA 3 programa. Rezultati su pokazali da je najmanja genetska udaljenost (16,7415) utvrđena između var. *cerasiforme* (žuti) i var. *cerasiforme* (crveni), a najveća (34,9859) između var. *pyriforme* i var. *grandifolium*. S druge strane, najveća genetska udaljenost (22,1446) ustanovljena je između podvrsta *subspontaneum* i *spontaneum*, a najveća (29,7147) između podvrsta *subspontaneum* i *cultum*. Na osnovu statističkih podataka urađeni su precizni dendogrami.

Ključne reči: *Lycopersicon esculentum* Mill; varijetet; SSR; genetička raznolikost