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Original scientific paper

CHARACTERIZATION OF A LOCUS *LECH13* IN DIFFERENT TOMATO VARIETIES USING FRAGMENT ANALYSES

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The microsatellite markers are routinely used to investigate the genetic structure of natural populations. The microsatellite polymorphisms are important for estimation diversity among varieties and for evaluation of the efficiency of microsatellite for establishing varieties relationships. The locus *LECH13* was tested in six tomato varieties in order to evaluate its usefulness in the genetic differentiation among six morphologically different tomato varieties. The fragment analyses were performed using the *Applied Biosystems* DNA analyzer. The number of detected alleles for the microsatellites locus *LECH13* was four in researched tomato varieties (134-136-138-146 bp). The allele138 bp was noticed only in *Lycopersicon esculentum* subsp. *spontaneum*var. *racemigerum*. The average PIC value for the locus *LECH13* was 0.3677 and it belongs to the group of modest informative markers. The present study showed that the locus *LECH13* could be used in the genetic differentiation of tomato varieties, but in combination with other polymorphic microsatellite loci.

Key words: DNA microsatellites, fragment analyses, locus *LECH13*, tomato, varieties

INTRODUCTION

The simple sequence repeats (SSRs) or DNA microsatellite markers are important molecular tools for the phylogenic estimations and determination of the genetic distance among different systematic categories. They are short tandem repeats (2-10 bp), middle repetitive, tandemly arranged, hypervariable DNA sequences distributed in the plant, animal and human genomes. According to Zane *et al*. [1] microsatellites are present in both coding and noncoding regions and are usually characterized by a high degree of length polymorphisms. The informativeness of microsatellites as genetic markers has already been shown with great success in several plant species [2]. According to He *et al.* [3], the allelic variation may be correlated with the number of repeats within a particular microsatellite locus. In other words, the repeat length may correlate with the polymorphism information content (PIC). The usually high variability of microsatellites might lead to inconsistencies due to the high chance of independently arising, equally sized alleles (homoplasies) [2]. Such microsatellites may generate polymorphisms useful for the analysis of genetic diversity and relationships within the genus *Lycopersicon*. When choosing new microsatellite loci for identification purposes or for studies on genetic variation, both the level of polymorphism and the scorability of the banding patterns are important [4]. Molecular marker must be very informative, especially in a crop like *Lycopersicon esculentum*, where genetic diversity seems very limited [5]. In our previous research precise dendrogram was created based on the data genetic distance among investigated tomato [6]. In this study, only locus LECH13 was in the focus.

The aim of the present study was to survey the applicability of the locus *LECH13* in genetic differentiation among six morphologically different tomato varieties of *Lycopersicon esculentum* Mill.

EXPERIMENTAL SECTION

Plant material

Six tomato varieties of *Lycopersicon esculentum* Mill. (var. *grandifolium* from subsp. *cultum*; var. *cerasiforme* – red and yellow, var. *pruniforme* and var. *pyriforme* from subsp. *subspontaneum*; and var. *racemigerum* from subsp. *spontaneum*) were involved in this research. There are many classifications of the genus *Lycopersicon*, but in this research was used classification by Brezhnev [7]. A comparison between the used nomenclature and nomenclature of Peralta *et al*. [8] is presented in Table 1.

Table 1. Comparison between different tomato nomenclatures

Tomato names (Peralta *et al.*)	Lycopersicon equiualent
Solanum habrochaites S. Knapp and D.M Spooner	Lycopersicon hirsutum Dunal
Solanum peruvianum L.	part of Lycopersicon peruvianum (L.) Miller
Solanum arcanum Peralta	part of Lycopersicon peruvianum (L.) Miller

The plant material was obtained from the GeneBank of the Agricultural Institute in Skopje

DNA isolation and PCR conditions

Fresh leaves were collected from ten individual plants per each variety. DNA was isolated using Promega's Wizard ® Genomic DNA purification kit. Also, DNA was extracted from pooled seeds (received from the fruits of 10 individual plants) of each variety using modified CTAB method [9–11]. The quality of the isolated DNA was examined by running on 0.8 % agarose gel. The optimization of the PCR conditions for amplification of the locus *LECH13* was carried out using appropriate primers (Operon, Huntsville, AL). Some general data for the locus *LECH13* and appropriate primer are given in Table 2. The PCR products were visualized by running on 2 % agarose gel, stained with ethidium bromide and photographed under UV light by using a G-Box system (Sygene).

Table 2. General data for microsatellite locus *LECH13* and primers used in this study

Locus	Repeat motif	Primer sequences (5'-3')
LECH13	$(TA)_{6\text{-}1}(GA)_4$	F: M13-taa caa tca aaa gaa ctt cgc
		R:atc ccc tta ttg att aca tcc

F - Forward primer (5'-3')
R - Reverse primer (5'-3')
M13 tail: 5'-cac gac gtt gta aaa cga c-3'

The DNA isolation and optimization of the PCR conditions were done in the Laboratory for biochemistry and molecular biology within the Department of Biochemistry and Genetic Engineering at the Faculty of Agricultural Sciences and Food – Skopje [12].

Data analyses

The fragment analyses were realized using the *Applied Biosystems* DNA analyzer (*ABI 3130*) and *GeneMapper®Software program* (v. 3.2). The data were analyzed using the specific program *Power Marker Software* (v. 3.25).

RESULTS AND DISCUSSION

The microsatellites are specific for each individual genome or species. They were used to evaluate genetic diversity and relationships within the genus *Lycopersicon*. The locus *LECH13* was used in many research, but in different tomato cultivars and accessions [2–4, 12, 14]. The main objective of this work was to examine the potential of the locus *LECH13* in genetic differentiation among six morphologically different tomato varieties of *Lycopersicon esculentum* Mill., received from Gene Bank of the Agricultural Institute in Skopje.

The analyzed microsatellite primer set gave good amplification across the six tomato varieties and was used for the fragment analysis. The results from fragment analysis were shown as electropherograms of homozygous (Figure 1 a) and heterozygous samples (Figure 1 b and c).

A. very important part of the fragment analyses is an interpretation of the obtained electropherograms. Namely, the additional problem of the

fragment analyses can be a determination of the peak (or peaks). This step is important for the relevant conclusion regarding the homozygous or heterozygous profile of the samples. In that sense, it is necessary to be careful in electropherograms' analyzing because further statistical analyses are based on these results.

It is important to select the major allelic peaks and to ignore the stutter peaks. The stutter peaks are small peaks that appear before, rarely after major allelic peaks and are side effects during the amplification of the microsatellite loci. They could be recognized according to their sizes and locations. It is recommended to take in consideration peaks higher than 100 RFU (relative fluorescence units) and lower than 2000 RFU. The peaks lower of 100 RFU must be interpreted very carefully.

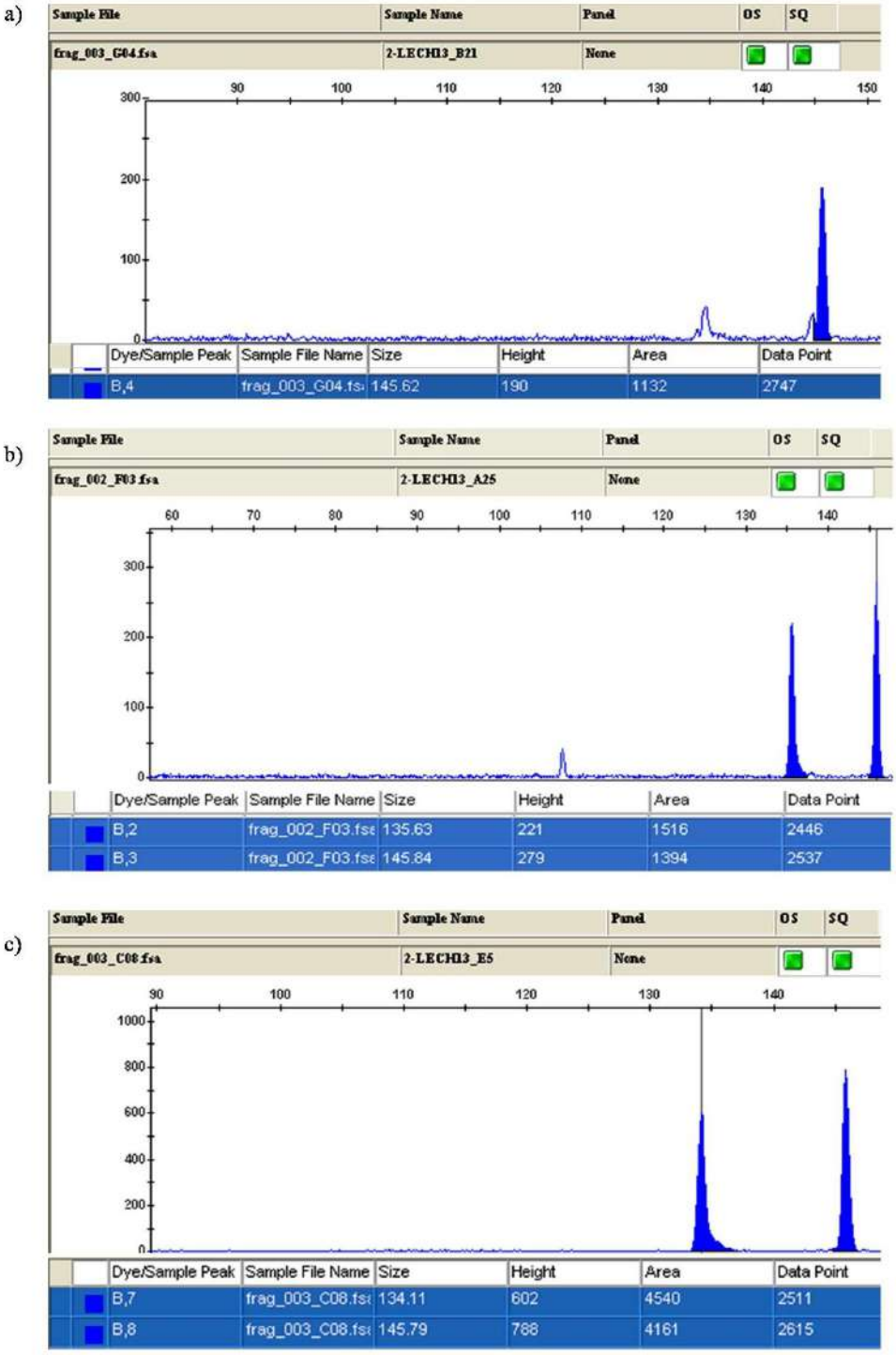

Figure 1. Electropherograms of locus *LECH13*: a) homozygous; b) and c) heterozygous

The fragment analyses of the locus *LECH13* (Figure 1) showed 4 allelic variants (134-136-138-146 bp). The allelic variants and their frequencies are presented in Figure 2. One of these alleles (138 bp) was specific for *Lycopersicon esculentum* subsp. *spontaneum* var. *racemigerum*.

For the same locus Smulders *et al.* [4] noticed 2 different alleles in researched tomatoes (124-128 bp), while Bredemeijer *et al.* [12] found only one allele (126 bp). Alvarez *et al.* [2] detected 5 different alleles among the investigated tomatoes (124-126-128-130-132 bp), and only one of them was a specific allele. According to He *et al.* [3], only one allele was noticed on this microsatellite locus. Garcia-Martinez *et al.* [14] found two alleles (124-128 bp) on the same locus.

It can be concluded that data, related to allele number and size, obtained in this research were different from previous results published by Smulders *et al.* [4], Bredmeijer *et al.* [13], Alvarez *et al.* [2], He *et al.* [3], Garcia-Martinez *et al.* [14]. One of the reasons for this could be the different plant material used in each research. For instance, Bredmeijer *et al.* [13] and He *et al.* [3] researched only cultivated tomato accessions, whereas in this study, tomato varieties that belong to subsp. *cultum*, subsp. *subspontaneum* and subsp. *spontaneum* were included.

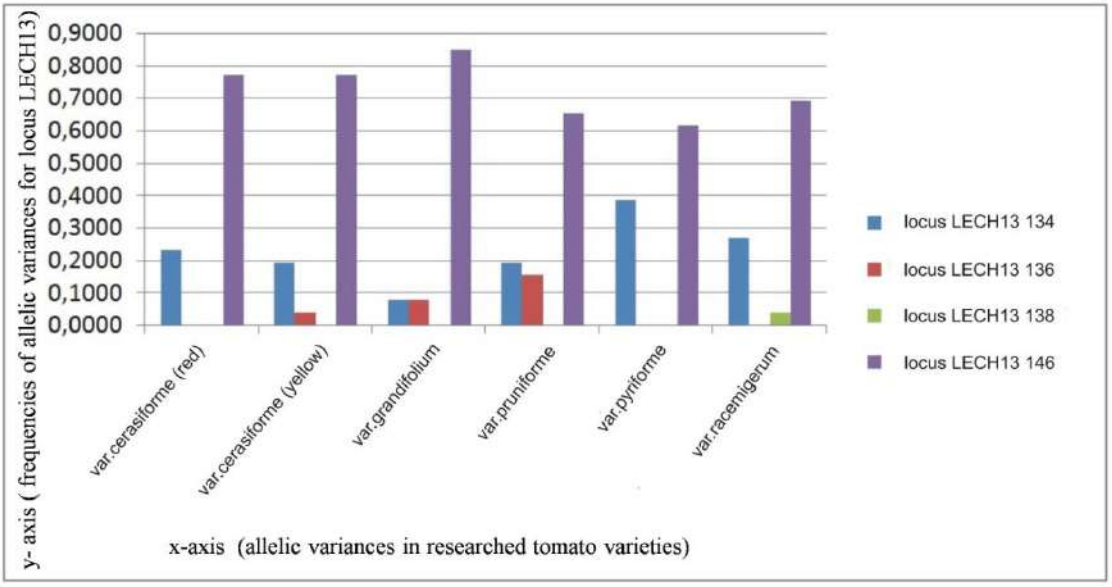

Figure 2. Allelic variances and their frequencies for locus *LECH13*

The difference in allele size could be related to the methodological approach. Namely, different DNA analysers, as well and different work conditions on the same DNA analyser (for ex. different capillary length, different type of polymer) could be the reason for receiving such differences in allele size. This means that doing analyses on the same DNA analyser and in the same working conditions (for ex. same capillary length, same type of polymer) is the best approach.

From the data presented in Figure 2, it can be concluded that the allelic variants in size of 134 and 146 bp appeared on the locus *LECH13* among all researched varieties, while allele of 136 bp is present only in *Lycopersicon esculentum* subsp. *subspontaneum* var. *cerasiforme* (yellow), *Lycopersicon esculentum* subsp. *cultum* var. *grandifolium* and *Lycopersicon esculentum* subsp. *subspontaneum* var. *pruniforme*. The allele (138 bp) was noticed only in DNA isolated from the seed of *Lycopersicon esculentum* subsp. *spontaneum* var. *racemigerum*. This allele was not noticed in the fragment analyses of DNA received from leaves of *Lycopersicon esculentum* subsp. *spontaneum* var. *racemigerum*, neither in the fragment analyses of DNA received from seed, respectively from leaves of the other varieties. This conclusion is probably due to the fact that in the fragment analyses of DNA from leaves of *Lycopersicon esculentum* subsp. *spontaneum* var. *racemigerum*, were not included plants that contain this allele, while the seed material was mixed.

Based on the obtained data, it could be concluded that the individual approach, using DNA isolation from individual plants, is better for fragment analyses. If we decide to use DNA isolated from

pooled seeds, probably we will have to include a much bigger number of samples.

In the researched varieties, the highest allele frequency was found for the allelic variant of 146 bp, and its values were: (0.7692) for *Lycopersicon esculentum* subsp. *subspontaneum* var. *cerasiforme* (red) and *Lycopersicon esculentum* subsp. *subspontaneum* var. *cerasiforme* (yellow), (0.8462) for *Lycopersicon esculentum* subsp. *cultum* var. *grandifolium*, (0.6538) for *Lycopersicon esculentum* subsp. *subspontaneum* var. *pruniforme*, (0.6154) for *Lycopersicon esculentum* subsp. *subspontaneum* var. *pyriforme* and (0.6923) for *Lycopersicon esculentum* subsp. *spontaneum* var. *racemigerum*.

For the locus LECH13, average observed heterozygosity (Ho = 0.5513) was higher than average expected heterozygosity (He = 0.4229), meaning the increased level of heterogeneity in the researched tomato varieties. Also, the observed heterozygosity was higher than the expected heterozygosity and it indicates a high level of allogamy.

The informativeness of polymorphic DNA markers could be quantitatively measured by a statistic called the polymorphism information content or PIC. In the researched tomato varieties, the average PIC value for the locus *LECH13* was 0.3677. According to the classification of Botstein *et al.* [15], the locus *LECH13* showed modest informativeness for all researched varieties.

The genetic differentiation test in the researched tomato varieties showed minor differentiation for the locus *LECH13* (0.0256). On the other hand, in the estimated tomato subspecies, this locus showed modest differentiation (0.0896) [16].

The present data show that this microsatellite locus gave amplification and polymorphism across six tomato varieties. However, data from a number of microsatellite loci will have to be combined to provide a unique DNA profile for individual varieties. Therefore, a combination of the locus *LECH13* with other more polymorphic microsatellite loci will be necessary to allow distinguishing tomato varieties.

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КАРАКТЕРИЗАЦИЈА НА *LECH13*-ЛОКУСОТ ВО РАЗЛИЧНИ ВАРИЕТЕТИ ДОМАТИ СО УПОТРЕБА НА ФРАГМЕНТ-АНАЛИЗИ

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Микросателитските маркери се користат рутински за испитување на генетската структура на природната популација. Микросателитските полиморфизми се важни за процена на разновидноста меѓу вариететите и за евалуација на ефикасноста на микросателитите во утврдување врски меѓу вариететите. *LECH13*-локусот беше тестиран кај шест вариетети на домати со цел да се оцени неговата корисност во генетската диференцијација меѓу шесте морфолошки различни вариетети на домати. Фрагмент-анализите беа изведени со ДНК-анализатор на *Applied Biosystems*. Во испитаните сорти домати, на микросателитскиот локус *LECH13* беа забележани четири алелни варијанти (134-136-138-146 bp). Алелот (138 bp) беше забележан само кај *Lycopersicon esculentum* subsp. *spontaneum* var. *racemigerum*. Просечната вредност на PIC за *LECH13*-локусот беше 0.3677 и тој припаѓа на групата умерено информативни маркери. Ова истражување покажа дека *LECH13*-локусот може да се користи во генетска диференцијација на вариетети на домати, но во комбинација со други полиморфни микросателитски локуси.

Клучни зборови: ДНК-микросателити; фрагмент-анализи; *LECH13*-локус; домат; вариетети