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USEFULNESS OF A LOCUS LEEF1Aa IN THE GENETIC DIFFERENTIATION OF TOMATO VARIETIES

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Abstract

The molecular techniques provide new possibilities to characterize advanced genetic materials for registration purposes and for the protection of breeders' rights. The microsatellites appear as suitable molecular markers due to their highly polymorphic character. Such microsatellites may generate polymorphism useful for the analysis of genetic diversity and relationships within the genus *Lycopersicon*. The focus of the following study was usefulness of the locus LEEF1Aa in the genetic differentiation among six morphologically different tomato varieties of *Lycopersicon esculentum* Mill. The fragment analyses were done using *Applied Biosystems* DNA analyzer (*ABI 3130*) and *GeneMapper®Software program*. The obtained data were analyzed using the specific program *Power Marker Software*. The number of detected alleles for the microsatellites locus LEEF1Aa was six in estimated tomato varieties (219-221-223-225-227-229bp). The allele with the length of 229 bp was noticed only in *Lycopersicon esculentum* subsp. *cultum* var. *grandifolium*, while the alleles (221, 225 and 227 bp) in 4 varieties, the allele of 219 bp in 3 varieties and the allele of 223 bp in 2 varieties. The average PIC value for the locus LEEF1Aa was 0.7552 and it belongs to the group of high informative markers. Obtained results showed that the locus LEEF1Aais good choice for genetic differentiation of tomato varieties in combination with other polymorphic microsatellite loci.

Keywords: DNA microsatellites, fragment analyses, locus LEEF1Aa, tomato.

Introduction

Molecular biology is a powerful tool to study genetic diversity, which allows a better understanding of the relationships between species within the same genus, successful taxonomic classification, and greater ability to identify species and cultivars (Aguirre et al. 2017). A number of molecular marker technologies exist, each with different advantages and disadvantages. Molecular markers have great potential to identify the structure and genetic diversity of accessions (Raveendar et al. 2016). According to Suresh et al. (2014) genetic diversity analysis is important for collections, conservations and sustainable utilization of Genbank accessions. The tomato is one of the most important vegetable crops globally. It is a dicot species belonging to the family of *Solanaceae*. Different types of DNA markers were used to estimate the genetic diversity and phylogenetic relationship among tomato genotypes (Klein-Lankhorst et al. 1991, Kwon et al. 2009, Geethanjali et al. 2011). Microsatellites are valuable as molecular markers, particularly for studies for closely related individuals. Microsatellites also known as simple sequence repeats (SSRs), or simple sequence length polymorphisms, consist of tandemly repeated motifs of 2 to 6 bp and are a common feature of most eukaryotic genomes. SSRs markers have several advantages. They are co-dominant, meaning that heterozygous can be discerned from the homozygous. The markers are easily automated and it is possible to multiplex several markers with non-overlapping size ranges on a single electrophoresis run. The obtained results are highly reproducible. Many studies have described the application of SSRs to reveal polymorphisms in tomatoes (Smulders et al. 1997, He et al. 2003, Villalta et al. 2005,Garcia-Martinez et al. 2006, Mazzucato et al. 2008, Geethanjali et al. 2010, Geethanjali et al. 2011). The informative amount of DNA markers can be quantitatively measured statistically by

means of PIC (polymorphism information content). The locus LEEF1Aa was included in the research studies of Arens et al. (1995), Smulders et al. (1997), Bredemeijer et al. (1998), Villalta et al. (2005), Garcia-Martinez et al. (2006), Mazzucato et al. (2008). The aim of present study was to evaluate the potential of the locus LEEF1Aa in genetic differentiation among six different tomato varieties of *Lycopersicon esculentum* Mill.

Material and methods

In this research, the plant material was obtained from the GeneBank of the Agricultural Institute in Skopje.Six morphologically different 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 used. The DNA isolation and optimization of the PCR conditions were performed 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 (Miskoska – Milevska et al. 2012). The DNA isolation from fresh leaves was performed using Promega's Wizard ® Genomic DNA purification kit. The leaves were collected from ten individual plants per each variety. Also, DNA isolation was donefrom pooled seeds. The DNA isolation from seeds was performed using modified CTAB method (Doyle and Doyle 1987, Cullings, 1992, Miskoska – Milevskaet al. 2011). The quality of the isolated DNA was checked by running it on 0.8% agarose gel. The optimization of the PCR conditions for amplification of the locus LEEF1Aawas done using appropriate primers, produced by Operon, Huntsville, AL. Some general data for the locus LEEF1Aa and appropriate primer pair are showed in Table 1 (Miskoska-Milevska et al. 2012).The PCR products were visualized by running them on 2% agarose gel, stained with ethidium bromide and photographed under UV light by using G-Box system (Sygene).

Table 1. General data for microsatellite locus LEEF1Aa and primers used in this study

Locus	Repeat motif	Primer sequences (5'-3')
LEEF1	$Aa(TA)_8(ATA)_9$	F: M13-aaa taa tta gct tgc caa ttg
		R: ctg aaa gca gca aca gta ttt

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

The fragment analyses of the PCR products were performed on *Applied Biosystems* DNA analyzer (*ABI 3130*) using *GeneMapper®Software program*. The data analyzing was done by the specific program *Power Marker Software*.

Results and discussion

The analysed microsatellite primers gave amplification across all researched tomato varieties and were used for fragment analyses. The fragment analyses of the locus LEEF1Aa detected six allelic variances (219-221-223-225-227-229 bp)and were presented in the form of electropherograms (Fig. 1 and Fig. 2).One of these alleles (229 bp) was specific for *Lycopersicon esculentum* subsp. *cultum* var. *grandifolium*. For locus LEEF1Aa, Arens et al. (1995) detected six alleles (193-213 bp), even if they expected allelic variance in size 131 bp. According to Arens et al. (1995) generation of products which exceeded the expected sizes could be due to the deletions in the original sequences, or insertions in the cultivars used in their study. But, to be resolved this amplification should be performed on the cultivars from which the sequences were derived. Smulders et al. (1997) reported 8 different alleles in researched tomatoes, while Bredemeijer et al. (1998) detected 7 different alleles (198-200-202-204-206-208-213 bp). Only one allele (200 bp) was noticed by Villalta et al. (2005), while Garcia-Martinez et al. (2006) found 10 alleles in size range from 165 to 226 bp. The biggest number of alleles (13) was detected by Mazzucato et al. (2008). Many factors can be the reason for the differences in allele number and size, between this research and the researches mentioned above. Firstly, different plant material can be reason for that. In this research tomato

varieties from subsp. *spontaneum*, subsp. *subspontaneum* and subsp. *cultum* were used, while Bredemeijer et al. (1998) and Arens et al. (1995) included only cultivated tomato accessions. Also, Smulders et al. (1997) and Mazzucato et al. (2008) worked on cultivated and wild tomatoes. A collection of traditional tomato cultivars was studied by Garcia-Martinez et al. (2006). The second reason for the differences in allele number and size could be the methodological approach. Working on same DNA analyser with the same working conditions is the best option (Miskoska-Milevska et al. 2017).

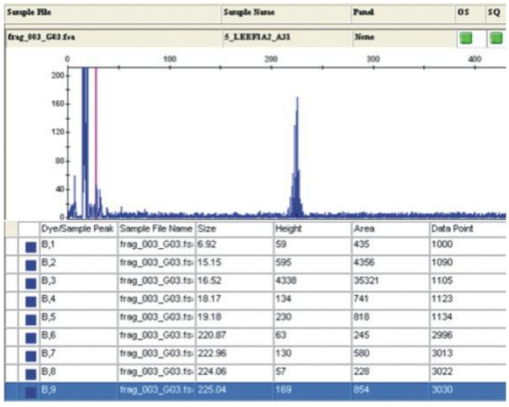

Fig. 1.Electropherograms of locus LEEF1Aa

The frequencies of allelic variances are presented in Fig. 3. The allelic variant of 219 bp was noticed for the locusLEEF1Aa in *Lycopersicon esculentum* subsp. *subspontaneum* var. *cerasiforme* (yellow), *Lycopersicon esculentum* subsp. *subspontaneum* var. *Pruniforme* and *Lycopersicon esculentum* subsp. *subspontaneum* var. *pyriforme.* The allelic variant in size of 221 bp was found for the locusLEEF1Aa in *Lycopersicon esculentum* subsp. *cultum* var. *grandifolium, Lycopersicon esculentum* subsp. *subspontaneum* var. *pruniforme, Lycopersicon esculentum* subsp. *subspontaneum* var. *pyriforme* and *Lycopersicon esculentum* subsp. *spontaneum* var. *racemigerum.*

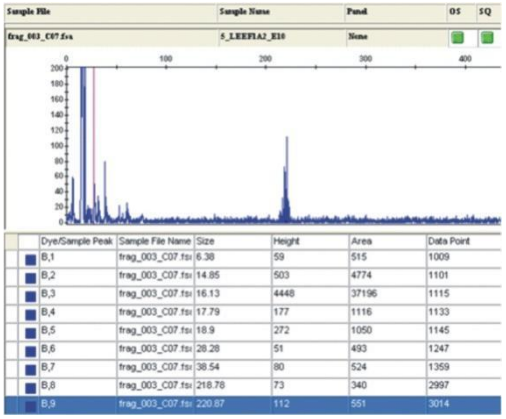

Fig. 2. Electropherograms of locus LEEF1Aa

The allele of 223 bp appeared on the locusLEEF1Aa in *Lycopersicon esculentum* subsp. *subspontaneum* var. *cerasiforme* (red) and *Lycopersicon esculentum* subsp. *spontaneum* var. *racemigerum.* The allelic variant in size of 225 bp was noticed for the locusLEEF1Aa across all analysed tomato varieties, with exception of *Lycopersicon esculentum* subsp. *subspontaneum* var. *pruniforme* and *Lycopersicon esculentum* subsp. *subspontaneum* var. *pyriforme.* The allele of 227 bp appeared on the locusLEEF1Aa across all analysed tomato varieties, only in *Lycopersicon esculentum* subsp. *subspontaneum* var. *cerasiforme* (red) and *Lycopersicon esculentum* subsp. *subspontaneum*

var. *pyriforme* was not found. The allelic variant of 229 bp was detected for the locusLEEF1Aa only in *Lycopersicon esculentum* subsp. *cultum* var. *grandifolium*.

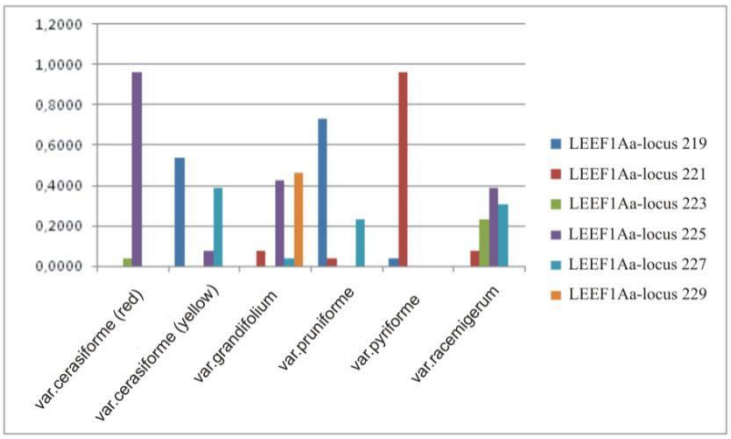

Fig. 3. Allelic variances and their frequencies for locus LEEF1Aa

In *Lycopersicon esculentum* subsp. *subspontaneum* var. *cerasiforme* (red) and *Lycopersicon esculentum* subsp. *spontaneum* var. *racemigerum* was detected the biggest allele frequency for allelic variant of 225 bp and its values were 0.9615 and 0.3846 respectively. For *Lycopersicon esculentum* subspvar. *Cerasiforme* (yellow) and *Lycopersicon esculentum* subsp. *subspontaneum* var. *pruniforme,* the biggest allele frequency was found for allele of 219 bp and its values were 0.5385 and 0.7308 respecively. In *Lycopersicon esculentum* subsp. *cultum* var. *grandifolium*, the biggest allele frequency was noticed for allelic variant of 229 bp and its value was 0.4615. For *Lycopersicon esculentum* subsp. *subspontaneum* var. *pyriforme,* the biggest allele frequency was found for allelic variant of 221 bp and its value was 0.9615 (Fig. 3). The average observed heterozygosity for the locus LEEF1Aa (0.1026) was lower than average expected heterozygosity (0.7872).This indicates on reduced level of heterogeneity in the analyzed tomatoes (Table 2). PIC-test determines informativeness of polymorphic DNA microsatellite loci and it is very important in analyses of the usefulness of microsatellite loci. In the researched tomato varieties average PIC value for the locus LEEF1Aa was 0.7552 (Table 2) and according to classification of Botstein et al. (1980), this locus belongs to the group of high informative markers.

Table 2. Genetic variability and polymorphism of locus LEEF1Aa in the researched tomato varieties

Locus	Number of genotypes	Number of alleles	*He*	*Ho*	PIC
LEEF1Aa	9.0000	6.0000	0.7872	0.1026	0.7552

He – expected heterosigosity;*Ho* – observed heterosigosityPIC-test for determination of informativeness for analysed DNA microsatellite locus

The genetic differentiation test in the investigated tomato varieties showedmajorgenetic differentiation for the locus LEEF1Aa (0.5006). Also, this test presentedmajordifferentiation for the locus LEEF1Aaon subspecies level (0.6167) (Miskoska-Milevskaet al. 2015).

Conclusions

Obtained results indicated that the locus LEEF1Aa gave amplification across all estimated tomato varieties. The number of detected allelic variants for this microsatellite locus was six in the researched tomato varieties. Only one specific allele of 229 bp in *Lycopersicon esculentum* subsp. *cultum* var. *grandifolium* was found. Received data showed that this microsatellitelocus isgood a choice for genetic differentiation of tomato varietiesin combination with other polymorphic microsatellite loci.

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