GENETIC ANALYSIS OF SOME TOMATO (*SOLANUM LYCOPERSICUM L*) GENOTYPES BY TBP AND SCOT MARKER SYSTEMS

ANALIZA GENETICĂ A UNOR GENOTIPURI DE TOMATE (*SOLANUM LYCOPERSICUM L*) UTILIZÂND SISTEMELE DE MARKERI TBP ŞI SCOT

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Abstract *Economically, the cultivated tomato (Solanum lycopersicum L). is one of the most important vegetables grown and consumed for its fruit all over the world. Majority of commercial cultivars of the tomato have been developed through phenotypic selection and can be easily confused because have similar morphological traits. Therefore, it is necessary to create fast, sensitive, and reliable methods for the tomato commercial cultivars authentication. Intended for that, in this study, were evaluated nine tomato cultivars (Kristinica, Florina, Andrada, Buzău-1600, Buzău-47, Argeș-11, Argeș-20, Ștefănești-24 and Ștefănești-22) at molecular level with two markers systems: TBP (tubulin-based polymorphism) that relies on the presence of intron-specific DNA polymorphisms of the plant β-tubulin gene family and six start codon-targeted (SCoT) markers. TBP analyzes, for beta tubulin gene, was performed for the both introns. The results showed identical electrophoretic profiles, consequently, no polymorphism was observed at the genotypes analyzed. Molecular assay with SCoT markers (SCoT2, SCoT13, SCoT16, SCoT20, SCoT24 and SCoT28) revealed a low level of polymorphism, although, SCoT markers generated between 7 bands (SCoT28) and 12 bands (SCoT24), polymorphic bands were obtained only with SCoT13 and SCoT28. A distinct PCR product of 600 bp was identified at cultivar Ștefănești -24 with SCoT 13 and with the marker -SCoT28 a distinct product of 1100 bp was observed at the genotypes: Andrada, Buzău- 1600 and Buzău- 47. These results open perspectives for tomato commercial cultivars authentication.*

Key words: tomato, SCoT markers, beta tubulin gene

Rezumat *Din punct de vedere economic, tomatele (Solanum lycopersicum L.) sunt printre cele mai importante legume, cultivate și consumate pentru fructele lor, în toată lumea. Majoritatea soiurilor comerciale de tomate au fost obținute prin selecție fenotipică și se confundă ușor între ele, deoarece au trăsături morfologice similare. Prin urmare, este necesar să se creeze metode rapide, sensibile și sigure pentru autentificarea soiurilor comerciale de tomate. Pentru această direcție, în acest studiu au fost evaluate nouă soiuri de tomate (Kristinica, Florina, Andrada, Buzău-1600, Buzău-47, Argeș-11, Argeș-20, Ștefănești-24 și Ștefănești-22) la nivel molecular cu două sisteme de markeri:*

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TBP care se bazează pe prezența polimorfismelor ADN, intron-specifice, ale familiei de gene pentru β-tubulină și șase markeri de tip SCoT care țintesc codonul start. Analizele TBP, pentru gena β-tubulinei, au fost efectuate pentru ambii introni. Rezultatele au arătat profile electroforetice identice, prin urmare, nu s-a observa polimorfism la genotipurile studiate. Analizele moleculare cu markerii SCoT (SCoT2, SCoT13, SCoT16, SCoT20, SCoT24 și SCoT28) au relevat un nivel scăzut de polimorfism, deși markerii SCoT au generat între 7 (SCoT28) și 12 benzi (SCoT24), benzi polimorfe au fost obținute numai cu SCoT13 și SCoT28. Un produs PCR distinct de 600 pb a fost identificat la soiul Ștefănești -24 cu markerul SCoT 13 iar cu markerul SCoT28 s-a observat un produs distinct de 1100 pb la genotipurile: Andrada, Buzău-1600 și Buzău-47. Aceste rezultate deschid perspective pentru autentificarea soiurilor comerciale de tomate.

Cuvinte cheie: tomate, markeri ScoT, gena pentru beta tubulină

INTRODUCTION

Tomato (*Solanum lycopersicum L.*) is an important genus in the *Solanaceae* family and the second most consumed vegetable crop in the world. Today are known almost 10,000 tomato cultivars, but the general tendency towards genetic and ecological uniformity imposed by the development of modern agriculture is drastically reducing the genetic diversity of tomato (Castellana *et al.*, 2020).

Many varieties can be easily confused because they have similar morphological traits, therefore, it is necessary to create fast and reliable methods for the tomato cultivars authentication.

Methodologies based on genetics and molecular biology are attracting great interest due to their applicability in identifying and differentiating between varieties (Karihaloo, 2015).

Tubulin-Based Polymorphism (TBP) is a molecular marker system based on the presence of intron-specific DNA polymorphisms of the plant β-tubulin gene family and belong to conserved DNA and gene family-based markers (CDMs) group (Poczai *et al.*, 2013). The TBP technique or cTBP or hTBP, depending on which intron or combination of introns is used as a marker (Breviario *et al.*, 2007; Braglia *et al.*, 2010; Galasso *et al.*, 2011; Poczai *et al.*, 2013) relies on an exon-primed intron-crossing (EPIC) PCR reaction.

Several papers have previously demonstrated that any of these TBP techniques is well suitable for genotyping new or neglected species (Galasso *et al.*, 2015; Braglia *et al.*, 2020).

Start codon targeted (SCoT) marker system belong to targeted fingerprinting markers (TFMs) group (Poczai *et al.*, 2013). This method is based on the observation that the short-conserved regions of plant genes are surrounded by the ATG translation start codon (Collard and Mackill, 2009).

The purpose of this paper, is thus, to provide experimental information for a vaster appreciation of the TBP and SCoT markers systems in the context of the tomato genetic diversity study and/or varieties authentication.

MATERIAL AND METHOD

The plant material consisted from young leaves of nine tomato cultivars (Kristinica, Florina, Andrada, Buzău-1600, Buzău-47, Argeș-11, Argeș-20, Ștefănești-24 and Ștefănești-22) and was provided by University of Agriculture and Veterinary Medicine, Bucharest, Research Center for the Study of the Quality of Agri-Food Products-HORTINVEST.

DNA extraction - total genomic DNA was isolated from young leaves, with an extraction buffer based on CTAB, according to the protocol by Ciucă *et al*. (2020).

Molecular marker analysis - two marker systems: TBP and SCoT.

TBP technique - all three methods TBP, cTBP and hTBP were used. Primers were selected from Braglia *et al*. (2010) as shown in table 1. Amplification reactions were carried out in a total volume of 20 µl, which contained 30 ng of template DNA, 1x buffer DreamTaq Green PCR Master Mix (Thermo Scientific), 1.25 µM from each primer and 0,2 µl Enhancer solution (10X PCRs enhancer Solution-Invitrogen) in a ProFlex (Applied Biosystem) thermocycler system. Following the initial denaturation step 95°C for 3 min, the PCR consisted of 38 cycles at 95°C for 30 sec., 40 sec. at 55°C, 1.30 min at 72°C. The reactions were held at 20 °C after a final extension for 10 min at 72°C. After PCR amplification, the samples were loaded in 1.5% agarose gel (routine use), stained with ethidium bromide. A 100pb ladder (Cleaver Scintific-CSL100-3000bp) was used as a molecular size standard. Gels were visualized in a gel documentation system UVITEC HD6.

SCoT technique - primers (Collard and Mackill, 2009) used in this study are presented in table 1. PCRs were carried out in a total volume of 20 µl, with 30 ng of template DNA, 1x buffer DreamTaq Green PCR Master Mix (Thermo Scientific), 0.6µM primer in a ProFlex (Applied Biosystem) thermocycler system. Following the initial denaturation step 95°C for 3 min, the PCR consisted of 38 cycles at 95°C for 45 sec., 1 min at 48°C, 2 min at 72°C, and the final extension step for 10 min at 72°C. After PCR amplification, the samples were loaded in 1.5% agarose gel (routine use), stained with ethidium bromide.

Table 1

Primers used in this study

No	Primer name	Primer sequences	%GC
1	SCoT-2	CAACAATGGCTACCACCC	56
2	SCoT-13	ACGACATGGCGACCATCG	61
3	SCoT-16	ACCATGGCTACCACCGAC	56
4	SCoT-20	ACCATGGCTACCACCGCG	67
5	SCoT-24	CACCATGGCTACCACCAT	56
6	SCoT-28	CCATGGCTACCACCGCCA	67
7	TBP - Intron 1- Fex1	AAC TGG GCB AAR GGN CAY TAY AC	-
8	TBP - Intron 1- Rex1	ACC ATR CAY TCR TCD GCR TTY TC	-
9	cTBP - Intron 2 - Fin2	GAR AAY GCH GAY GAR TGY ATG	-
10	cTBP - Intron 2 - Rin2	CRA AVC CBA CCA TGA ARA ART G	-
11	hTBP - Intr.1-ex.2-intr.2 -Fex1	AAC TGG GCB AAR GGN CAY TAY AC	
12	hTBP - Intr.1-ex.2-intr.2 -Rin2	CRA AVC CBA CCA TGA ARA ART G	

RESULTS AND DISCUSSIONS

All three TBP markers used in this study could not differentiate a distinct profile for any of the nine tomato cultivars, although PCR products were

obtained. As an example, PCR products for hTBP ranged between 550-2250 bp (fig. 1). This means, at least for our tomato cultivars, the TBP method has no applicability in obtaining valuable information regarding genetic diversity and cultivar authentication.

These results could be due to a selective pressure caused by domestication and breeding resulted that determined a reduction in genetic diversity and a genetic bottleneck for agrobiodiversity in tomato. These results could be the first report regarding the TBP techniques on tomato. On the other hand, these TBP markers could be used to study local varieties, which are often referred to as "landraces".

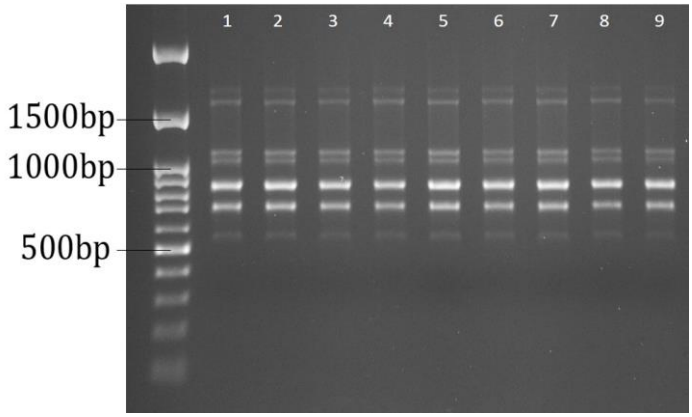

Fig. 1 hTBP technique with FEX1/RIN2 primers. Electrophoresis pattern: 100pb DNA ladder, 1. Kristinica, 2. Florina, 3. Andrada, 4. Buzău 1600, 5. Buzău 47, 6. Argeș 11, 7. Argeș 20, 8. Ștefănești 24, 9. Ștefănești 22

SCoT analysis used to study the genetic differences and uniqueness allele among the tomato cultivars was carried out with six SCoT primers. A total of 44 bands were generated.

The total bands per primer ranged from 7 (SCoT 28) to 12 (SCoT 24). The size of amplified products ranged from 270 to 3000 bp. Maximum number of polymorphic bands were obtained with SCoT 16 (tab. 2).

Table 2

SCoT markers - number of fragments and sizes

No	Primer	Total No fragments	No polymorphic fragments	Products size (bp)
1	ScoT-13	8	2	600-3000
2	ScoT-16	10	3	600-2500
3	ScoT-20	9	2	500-2000
4	ScoT-24	12	2	400-2900
5	ScoT-28	5	1	270-1250

Although polymorphism was revealed by the PCR analyses with all SCoT markers (except SCoT2 that revealed unclear profiles), clear polymorphic fragments between tomato cultivars was observed with SCoT 13 that revealed distinct aleles of 600 bp for cultivar Ștefănești 24 and 2000 bp for cultivars Florina, Andrada, Buzău 1600 and Ștefănești 24 (fig. 2). Also, primer SCoT28 generated a product of 1100 bp for cultivars: Andrada, Buzău 1600 and Buzău 47 (fig. 3).

Considering that SCoT13 generated a distinct unique allele for Ștefănești 24, this marker could be useful in the following studies for genetic diversity and tomato cultivars authentication.

The primers SCoT16, SCoT20 and SCoT24, although they have generated polymorphism, the polymorphic bands were a little unclear, meaning that the PCR conditions needs some modifications.

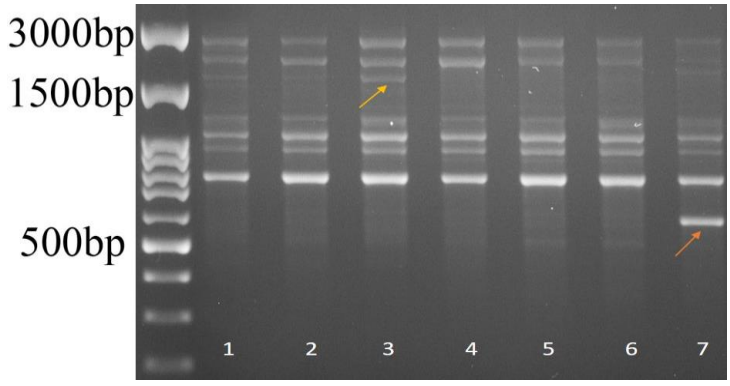

Fig. 2 Electrophoresis pattern obtained with primer SCoT 13: 100pb DNA ladder, 1. Florina, 2. Andrada, 3. Buzău 1600, 4. Buzău 47, 5. Argeș 11, 6. Argeș 20, 7. Ștefănești 24

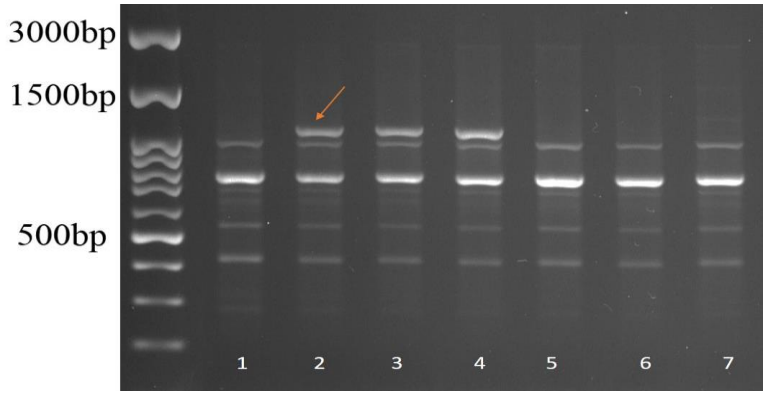

Fig. 3 Electrophoresis pattern obtained with primer SCoT 28: 100pb DNA ladder, 1. Florina, 2. Andrada, 3. Buzău 1600, 4. Buzău 47, 5. Argeș 11, 6. Argeș 20, 7. Ștefănești 24

CONCLUSIONS

1. Between the two markers systems used in this study only SCoT generated distinct polymorphic PCR products.

2. From the six SCoT markers used in this study only SCoT13 and SCoT28 markers generated polymorphic bands.

3. These results suggest that the SCoT markers system could be useful for tomato commercial cultivars authentication.

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