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Efficiacy of SCoT and ISSR marekers in assesment of tomato (*Lycopersicum esculentum* Mill.) genetic diversity

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Abstract

Two molecular marker systems, SCoT and ISSR were used for genetic diversity analysis of 10 Tomato (*Lycopersicum esculentum* L.) accessesions. Using 10 selected SCoT primers 83 bands were generated, of which 30 (36.14%) were polymorphic. 10 selected ISSR primers amplified 86 bands with 20 (23.25%) being polymorphic. Average PIC values for SCoT and ISSR marekers were 0.142 and 0.088 respectively. Mean RP values for SCoT and ISSR marekers were 1.88 and 1.55 respectively. The 10 accessesion were clustered into 3 major groups based on the SCoT analysis and 2 major groups based on the ISSR analysis with UPGMA. The PCA Analysis confirmed the results of the clustering. The results also demonstrate that the SCoT marker system is useful for identification and genetic diversity analysis of tomato accessesions. In general, SCoT molecular marker was informative than ISSR molecular system.

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Introduction

Tomato (*Lycopersicum esculentum* Mill.) is one of the most importat vegetables of night shade (solanaceae) family that consumed in diverse ways, including raw, as an ingredient in many dishes, sauces, salads, and drinks. The genome of this plant is one of the most investigated plant genomes (Foolad, 2007). Molecular markers have proven to be valuable tools in the evaluation of genetic variation both within and between species (Powell *et al.*, 1996). The analysis of genetic diversity and relatedness between or within different species, populations and individuals is a prerequisite towards effective utilization and protection of plant genetic resources (Weising *et al.,* 1995). In recent years, many new alternative and promising marker techniques have been developed in line with the rapid growth of genomic research (Gupta and Rustgi, 2004). There must be a set of polymorphic markers to evaluate relation among closely related speicies and varieties (Santalla *et al.*, 1998). Beacause of limitations come along with morphological and izozyme markers (Kumar *et al.*, 2009; Poehlman *et al.*, 2003) DNA-based molecular markers have been developed (Agarwal. 2008; Semagn, 2006). Among DNA-based molecular markers those who are simple and fast and don't use radiactive materials are in interest. ISSR marker because of many advantages is used by many reasercher in many plant genus and spicies (Pradeep Reddy *et al.*, 2002). Recently, a new DNA-based molecular marker system named SCoT have been emerged and announced by Collard and Mackill 2009. This marker use single primer as the forward and reverse primer. The anealing temprature of SCoT primers is set to 50 °C. They are dominant markers like RAPDs and could be used for genetic analysis, QTL mapping and bulk segregation analysis (Collard and Mackill, 2009). The SCoT molecular system has been successfully used in diversity analysis and diagnostic fingerprinting in potato, grape, peanut, *Dendrobium nobile* , *Cicer* and mango (Gorji *et al.*, 2011; Guo *et al.*, 2012; Xiong *et al.,* 2011; Bhattacharyya *et al.*, 2013; Amirmoradi *et al.*, 2012; Luo *et al.*, 2010). Molecular markers have been used extensively in tomato breeding. The most important uses have been: the study of molecular variability and phylogenetic relationships, the varietal identification, the marker-assisted selection, the map-based cloning of genes or QTLs, the construction of high-density maps and the construction of mapping populations (Foolad and Sharma 2005). By 2008, more than 2500 markers, including RFLP, EST (Expressed Sequence Tag polymorphism), SSR (Simple Sequence Repeat) and COS (Conserved Orthologue Set) were mapped on 12 tomato chromosomes (Frary *et al.,* 2005; Barone *et al.* 2009). A complete review of the applications of molecular markers in tomato can be found in Foolad and Sharma (2005).

Until now, there is no report base on SCoT marker application on tomato.The present study was conducted to evaluate the efficiacy of SCoT marker and to compare with ISSR molecular marker.

Materials and methodes

Plant Materials and DNA Extraction

Seed samples used in this study were prepared from Karaj Seed and Plant Research Institute and are listed in table1. The seeds were planted in pots with 15 cm diameter in a growth chamber at a constant temperature of 25°C at Islamic Azad University of Dezful. After 3 weeks, the leaves of plants grown enough to have a few fresh leaves were collected from each plant sample. In order to prevent deterioration, the samples were immediately transferred to a freezer (-20°C). Then, plant leaves were ground under liquid nitrogen to obtain a fine powder. Genomic DNA extracted from powdered leaves by method of Cortés *et al.* Purified total DNA was quantified and its quality was verified by spectrophotometer and gel electrophoresis. Each sample was diluted to 50 ng/µl with TE buffer (10mM Tris–HCl, pH 8.0 and 0.1mM EDTA, pH 8.0) and stored at 4 °C for further use.

PCR primers, materials and condition

Amplifications of SCoT and ISSR primers were performed in a 25µl reaction volume containing 2µl DNA (50 ng), 12.5µl Master Mix (DFS-Master Mix Blue, Ready For Gel 2X, Bioron corporation, Germany containing : 4 mM $MgCl_2$, 1.6 mM dNTPs, Ready For

Gel PCR Buffer, DFS-Tag DNA Polymerase.), 2µl of 10 µM primer and 8.5 µl of disitilled water. Amplification of SCoT primers was performed in a programmed thermocycler (Palm Cycler Corbet, Germany) with initial denaturation at 94°C for 3min, 36 cycles of denaturation at 94°C for 1min, primer annealing at 50°C for 1min, extension at 72°C for 1min, and final extension at 72°C for 3min. For Amplification of ISSR primers the following programe was used: initial denaturation at 94°C for 3min, 36 cycles of denaturation at 94°C for 45s, primer annealing was 48°C or 52°C depending on primer for 1min, extension at 72°C for 90s, and final extension at 72°C for 5min. Amplified products were electrophoresed in 1.5% agarose in 1× TBE buffer. The gels were stained with ethidium bromide and documented using gel documentation system (Bio-Rad, Hercules, California). Each experiment was repeated two times with each primer and those primers which gave reproducible fingerprints (DNA bands) were only considered for the data analysis. The set of ISSR and SCoT primers used in our investigation is shown in table 2.

Data Analysis

PCR-amplified SCoT and ISSR fragments detected on 1.5% gels were scored as absent (0) or present (1). Only clear, reproducible bands were scored. A dendrogram showing the genetic relationships between accessions, based on the unweighted pair-group method with arithmetic averages, was constructed using NYSYS-pc software version 2.02 (Rohlf , 1998). Genetic similarity among samples was evaluated by calculating the Jachard similarity coefficient and cluster analysis was performed using the UPGMA algorithm. Polymorphic Information Content of each of the analysed SCoT and ISSR was calculated according to:

PIC = 1-p2−q2 (Ghislain *et al*. 1999).

where p is frequency of present band and q is frequency of absent band. The BI index (Band Informativness) was calculated using the formula:

BI= 1-(2*|0.5-*p*|).

where *p* is the proportion of the occurrence of bands in the genotypes out of the total number of genotypes.Resolving power (Rp) each primer was calculated using the formula:

Rp= Σ bI.

Resolving power of primer was calculated according to Prevost and Wilkinson, 1999 where, bI = Band informativeness

Marker index was calculated as given in Varshney *et al*. 2005:

MI = PIC × EMR.

where, EMR = Effective multiplex ratio (E) is defined as the product of the total number of fragments per primer (n) and the fraction of polymorphic fragments(β).

EMR = nβ

n= total number of bands.β= total number of polymorphic bands. (Suman Tiwari *et al* 2013., Nagaraju *et al* 2001., Prevost and Wilkinson 1998). Principal Coordinate Analysis (PCA) was performed based on the matrix calculated from the marker data with the software package NTSYS-pc 2.02.

Results and discussion

ISSR Assay

In the present study we investigated the ability of 10 ISSR primers to generate polymorphic DNA fragments. The results of application of 10 ISSR primers are shown in table 3 and Figure 1. Except $(CT)_8A$ and $(AT)_8T$, all the other ISSR primers did produce bands. 10 selected ISSR primers amplified 86 bands with 20 (23.25%) being polymorphic. The maximum and lowest number of amplified bands were for $(AG)_8G$ with 16 bands and $(GT)_8T$ with 7 bands respectively. The values of polymorphism ranged from 11.11% $(TC)_8AGG$ to 53.84% $(GA)_8A$. The maximum and lowest PIC values were for $(GA)_8A$ (0.234) and $(AG)_8G$ (0.051) respectively. Average Rp value in ISSR marker was 1.55. The range of MI (Marker Index) was from 0 $(AC)_8CTT$) to 0.645 $(GA)_8A$. The maximum MI index was for $(GA)_8A$

0.645. Average MI value in ISSR marker was 0.122.

The UPGMA clustering algorithm from ISSR marker analysis grouped the 10 accessions into two main clusters (Fig. 2). The first cluster, group I, further divided into two sub-clusters. The first sub-cluster consisted of 3 accessions: Early Urbana Y, Early Urbana and Super Strain B. The second sub-cluster consisted of 2 accessions: Mobil and King Stone. The second major cluster, group II, consists of 5 accessions. This group also subdivided in two sub clusters. The first sub-cluster consisted of 2 accessions: Rio Grande and Peto Early ch. The second sub-cluster consisted of 3accessions: Primo Early, CAL JN3 and Peto Mech. The results of PCA analysis are shown in Fig. 3. It can clearly seen that accessions grouping are cosistent with dendogram obtained in Fig. 2. Tomato accessions grouped in two section based on PCA analysis.

Table 1. List of tomato specimens used in present study.

Column\Row	Code of Samples	Tomato Specimens
1	T1	Early Urbana Y
2	T2	Early Urbana
3	T3	Rio Grande
4	T4	Mobil
5	T5	Super Strain B
6	T6	King Stone
7	T7	CAL JN3
8	T8	Primo Early
9	T9	Peto Mech
10	T10	Peto Early ch

Table 2. Primer ID and sequences of both SCoT and ISSR used in the present study.

Primers ISSR		Primers SCoT	
Primer	Sequence 5´ to 3´	Primer	Sequence 5´ to 3´
(AC)8CTT	ACACACACACACACACCTT	SCoT1	GAAGAAGATGATGATGCC
(AG)8C	AGAGAGAGAGAGAGAGC	SCoT8	GATGGTGATGCAAAAGAG
(AG)8G	AGAGAGAGAGAGAGAGG	SCoT16	ATGTCATGCAGCACCCTC
(AG)8T	AGAGAGAGAGAGAGAGT	SCoT23	CCGACTTTGGGTATGCAC
(AT)8T	ATATATATATATATATT	SCoT30	AGACAGCTAAATGGGGTG
(CT)8A	CTCTCTCTCTCTCTCTA	SCoT31	CAAGGTCTTTAGGATGATCG
(CT)8T	CTCTCTCTCTCTCTCTT	SCoT34	CAATGCACGAATCGTGTC
(GA)8A	GAGAGAGAGAGAGAGAA	SCoT47	CATGCTGCTTGTGAGAGT
(GA)8T	GAGAGAGAGAGAGAGAT	SCoT57	AATGCTTGGAATCGGGTC
(TC)8AGG	TCTCTCTCTCTCTCTCAGG	SCoT70	ACTTATGGGCTGTAGAGG

SCoT Assay

The results of application of 10 SCoT primers are shown in tables 4 and Figure 4. Using 10 selected SCoT primers 83 bands were generated, of which 30 (36.14%) were polymorphic. The maximum and lowest number of amplified bands were for $SCoT_{31}$ with 15 bands and $SCoT_1$ and $SCoT_8$ with 5 bands respectively. The values of polymorphism varied from 20% to 73.33% . The highest and lowest PIC values were for SCoT30 (0.238) and $SCoT_{70}$ (0.073) respectively. Average RP value in SCoT marker was 1.88. The range of MI (Marker Index) was from 0 $SCoT_{23}$ to 8.066 $SCoT_{31}$. Average MI value in SCoT marker was 2.122.

The UPGMA clustering algorithm from ISSR marker analysis grouped the 10 accessions into two main clusters (Fig. 5). The first cluster, group I, further divided into two sub-clusters. The first sub-cluster consisted of 2 accessions: Early Urbana Y, Early Urbana. The second sub-cluster consisted of 3 accessions: Rio Grande, Mobil and King Stone. The second major cluster, group II, consists of 4 accessions. This group also subdivided in two sub

clusters. The first sub-cluster consisted of 3 accessions: CAL JN3, Primo Early, and Peto Mech. The second sub-cluster consisted of 1 accession: Primo Early. The third major cluster, group III, consists of 1 accession: Super Strain B. the results of SCoT Dendrogram and PCA analysis (Fig. 6) are consistent with each other. Based on PCA analysis, all tomato accessions were fall in three distinct groups.

Molecular markers can be used not only for estimating the genetic diversity of germplasm collections but also for distinguishing genotypes within populations. The reduction of genetic variation in tomato through domestication and breeding has resulted in the need for conservation, characterization, and utilization of genetic resources (Terzopoulos and Bebeli, 2008). Tomato accessions was divided in 2 groups based on ISSR marker and 3 groups based on SCoT marker. All of grouping was similar to each other except Super Strain B that biuld a seprate group in SCoT marker. Accessions named Early Urbana Y and Early Urbana beside accessions Peto Mech and Peto Early ch that are genetically close accessions fell in same group in two molecular markers. A possible explanation for the difference in resolution of SCoTs and ISSRs is that the two-marker techniques target different portions of the genome. These differences may also be attributed to marker sampling errors and/or the percent polymorphism detected by different markers, reinforcing the importance of the number of loci and their coverage of the overall genome in obtaining reliable estimates of genetic relationships among cultivars (Gajeraa *et al.*, 2010).

Table 3. Characteristics of ISSR banding profiles produced in 10 tomato accessasion.

ISSR Primers	GC%	Ta	NSB	NPB	Poly%	PIC	RP	RP Mean	EMR	MI PIC*EMR
(AC)8CT	47.36	52	8	0	0	0	0	0	0	0
(AG)8C	52.94	52	11	4	36.36	0.164	2.8	0.254	1.454	0.24
(AG)8G	52.94	52	16	3	18.75	0.051	1	0.062	0.562	0.03
(AG)8T	47	48	14	2	14.28	0.066	1.6	0.114	0.071	0.004
(CT)8T	47	48	7	2	28.57	0.086	0.8	0.114	0.571	0.048
(GA)8A	47	52	13	7	53.84	0.234	4.8	0.369	2.769	0.645
(GA)8T	47	52	8	1	12.5	0.053	0.6	0.075	0.125	0.006
(TC)8AGG	52.63	52	9	1	11.11	0.053	0.8	0.088	0.111	0.005

GC%= primer G-C content, T_a = Primer Anealing Temprature, NSB = Number of Scorable Bands, NPB = Number of Polymorphoic Bands, PIC= Polymorphic Information Content, RP = Resolving Power, EMR = Effective Multiplex Ratio, MI= Marker Index.

Table 4. Characteristics of SCoT banding profiles produced in 10 tomato accessasion.

SCoT Primers	GC%	Ta	NSB	NPB	Poly%	PIC	RP	RP Mean	EMR	MI PIC*EMR
SCoT1	47.36	50	5	2	40	0.157	1.6	0.184	0.15	0.8
SCoT 8	52.94	50	5	1	20	0.128	0.8	0.128	0.10	0.8
SCoT 16	52.94	50	6	2	33.33	0.163	1.8	0.163	0.11	0.666
SCoT 23	47	50	7	0	0	0	0	0	0	0
SCoT 30	50	50	10	5	50	0.238	3.4	0.238	0.856	3.6
SCoT 31	47	50	15	11	73.33	0.18	3.4	0.18	1.452	8.066
SCoT 34	47	50	10	2	20	0.292	4.8	0.292	1.430	4.9
SCoT 47	47	50	10	2	20	0.076	1.2	0.075	0.033	0.444
SCoT 57	47	50	6	3	50	0.113	0.8	0.113	0.169	1.5
SCoT $_{70}$	50	50	9	2	22.22	0.073	1	0.073	0.032	0.444

GC%= G-C content, T_a = Primer Anealing Temprature, NSB = Number of Scorable Bands, NPB = Number of Polymorphoic Bands, PIC= Polymorphic Information Content, RP = Resolving Power, EMR = Effective Multiplex Ratio, MI= Marker Index.

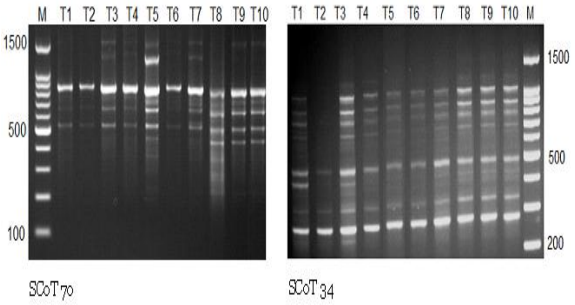

Fig. 1. Fingerprints of 10 tomato accessasions with two SCoT primers. M= 100bp DNA ladder. T_1 to T_{10} are listed in table 1.

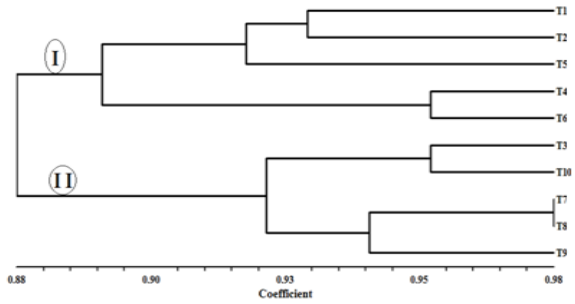

Fig. 2. Dendrogram of 10 tomato accessasions based on ISSR-marker clustered by UPGMA technique.

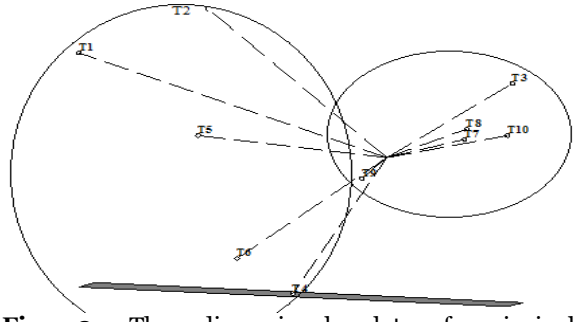

Fig. 3. Three-dimensional plot of principal coordinate analysis (PCA) of 10 accessasions of tomato using ISSR marker.

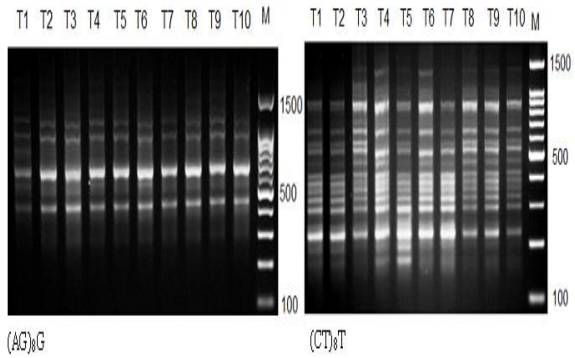

Fig. 4. Fingerprints of 10 tomato accessasions with two ISSR primers. M= 100bp DNA ladder. T_1 to T_{10} are listed in table 1.

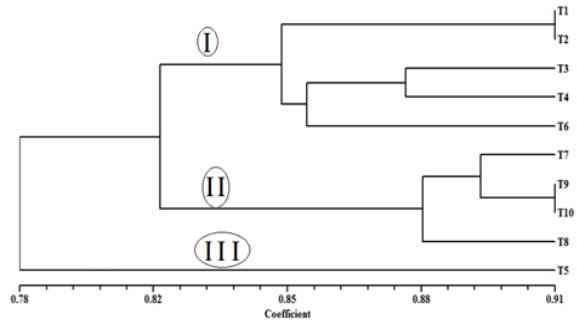

Fig. 5. Dendrogram of 10 tomato accessasions based on SCoT-marker clustered by UPGMA technique.

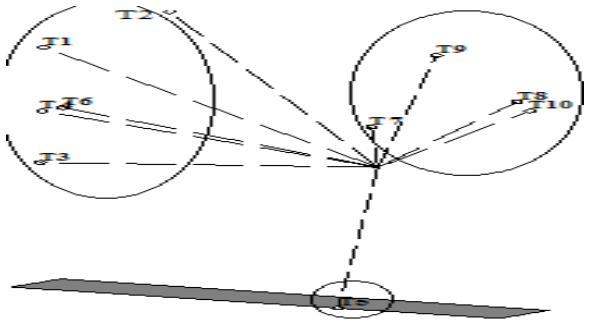

Fig. 6. Three-dimensional plot of principal coordinate analysis (PCA) of 10 accessasions of tomato using SCoT marker.

Conclusion

In the present study, we used 2 marker systems to evaluate tomato diversity. This is the first application of SCoT marker in the genus of *Lycopersicom* . Not all of the ISSR and SCoT primers were suitable. 2 of 10 ISSR primers didn't produced any bands. One SCoT and one ISSR primer produce only monomorph bands that are not suitable in genetic diversity analysis systems. Application of SCoT molecular marker in other plants such as mango (Luo *et al.*, 2011), potato (Gorgi *et al.*, 2011), *Cicer* (Amirmoradi *et al.*, 2012) showed that this marker is efficient than ISSR marker and this finding is consistenet with our results. The polymorphism percentage of SCoT marker in the tomato accessasions was 36.14% and this is lower than those reported in other plants such as as mango (76.19), *Cicer* (97.32%), grape (93.1%). This may be due to narrow genetic differences between these accessasions. Based on data obtained from these two molecular markers, SCoT marker was informative in all aspects such as polymorphism percentage, PIC values, Rp values, Marker indexing than ISSR marker.

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Abbreviations

PIC: Polymorphic Information Content

Rp: Resolving Power

PCA: Principle Component Analysis

SCoT: Start Codon Targeted

QTL: Quantitative Trait Loci

MI: Marker Index

UPGMA: Unweighted Pair Group Method of Arithmetic Means

SSR: Simple Sequence Repeat

ISSR: Inter Simple Sequence Repeat.