MOLECULAR CHARACTERIZATION AND GENETIC RELATIONSHIPS AMONG SOME TOMATO GENOTYPES AS REVEALED BY ISSR AND SCoT MARKERS

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Cultivated tomato (*Lycopersicum esculentum* L.) is a significant vegetable crop of economic importance and widely grown around the world (El-Hady *et al*., 2010). Botanically, it is a fruit and horticultural it is a vegetable. The popularity of tomato as fresh and processed crop has made it an important source of vitamins A and C in diets. In addition, it is a pre-eminent model system for genetic studies in plants.The genome of tomato plant is one of the most investigated plant genomes in but in the same time the cultivated tomato has limited variability mainly due to population bottlenecks occurred though domestication and evolution of modern cultivars (Foolad, 2007). Analysis of genetic relationships in crops is a prerequisite for crop breeding programs, as it serves to provide information about genetic variation (Mohammadi and Prasanna, 2003) Lack of genetic diversity can potentially lower the resistance of cropping systems to unknown or evolving pests, pathogens, or adverse environmental conditions. Therefore, sufficient information on the genetic diversity among tomato genotypes conserved in the gene banks is necessary for the development of effective breeding strategies.

There must be a set of polymorphic markers to evaluate relation among closely related species and varieties (Santalla and Davey, 1998). Because of limitations come along with morphological and isozyme markers (Andersen and Thomas, 2003), the use of molecular markers can facilitate tomato breeding by means of marker -assisted selection (MAS) to improveimportant agronomical traits such as yield, fruit quality and disease resistance. In several studies, the genetic diversity of tomato has been investigated using different molecular techniques. The most important uses have

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been the study of molecular variability and phylogenetic relationships, the varietal identification, the marker-assisted selection, the map-based cloning of genes or quantitative trait locus (QTLs), the construction of high-density maps and the construction of mapping populations (Foolad and Sharma, 2005). Despite of using different molecular markers to study genetic diversity in cultivated tomatoes, many of them identify limited level of polymorphism (Kochieva *et al*., 2002; Terzopoulos and Bebeli, 2008). Therefore, identification of more polymorphic molecular markers is important for tomato researches.

Inter simple sequence repeat (ISSR) markers are considered very useful in studies of genetic diversity, phylogeny, genomics and evolutionary biology (Reddy *et al*., 2002; Havlíčková *et al*., 2014). This marker combines most of the benefits of amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSR) analysis with the universality ofrandom amplification of polymorphic DNA (RAPD). This marker is a PCR-based technique, which involves amplification of DNA segment between adjacent and inversely oriented microsatellites (Singh *et al*., 2014). The technique uses microsatellites, usually 16-25 bp long, as primers. These primers can be di-, tri-, tetra- or penta-nucleotides. The usefulness of the ISSR markers for assessing genetic variability in the genus *Solanum* has been demonstrated (Aguilera *et al*., 2011; Edris *et al*., 2014; Shahlaei *et al*., 2014). Utilizing the ISSR markers to distinguish closely related morphotypes within tomato landraces would enable us to better use the full genetic potential of landraces in marker-assisted tomato improvement programs (Terzopoulos and Bebeli, 2008).

In recent years, many new alternative and promising marker techniques have been developed in line with the rapid growth of genomic researches (Gupta and Rustgi, 2004). With initiating a trend away from random DNA markers towards gene-targeted markers, a novel marker system called start codon targeted (SCoT) (Collard and Mackill, 2009) was developed based on the short-conserved region flanking the ATG start codon in plant genes. 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 nobileand* Cicer (Gorji*et al*., 2011; Xiong*et al*., 2011; Amirmoradi *et al*., 2012; Guo *et al*., 2012; Bhattacharyya *et al*., 2013). Objectives of the present study are as follows: (1) to determine the genetic diversity in tomato genotypes using SCoT and ISSR markers; (2) to determine the potential of these methodologies to generate polymorphic markers for detecting molecular variation in tomato; (3) to investigate whether SCoT and ISSR markers could be effectively used in determining genetic relationships among tomato genotypes compared to ISSR markers data; (4) to identi-

fy the relationships of different types of molecular fingerprinting based clustering of genotypes.

MATERIALS AND METHODS

Plant material and DNA isolation

Eight genotypes of tomato (*Lycopersicum esculentum* L.) were used in this study, collected from different countriesand considered in the current work (Table 1). The total genomic DNA was isolated from young leaves ofgreenhouse-grown plants according to the CTAB protocol (Doyle and Doyle, 1987).

Molecular marker analysis

ISSR analysis was performed as described by (Adawy *et al*., 2004) and (Hussein *et al*., 2006). Six ISSR primers (Table 2) were selected from different published papers to be employed in ISSR analysis. SCoT amplification was performed as described by (Collard and Mackill, 2009), using seven primers (Table 2), these primers were selected from published papers (Collard and Mackill, 2009; Xiong *et al*., 2011).

Data analysis

The similarity matrices were done using Gel works ID advanced software UVP-England Program. The relationships among genotypes as revealed by dendrograms were done using SPSS windows (Version 10) program. DICE computer package was used to calculate the pairwise difference matrix and plot the phenogram among cultivars (Yang and Quiros, 1993).

All the used primers were amplified distinct evaluable bands. The clear, reproducible alleles amplified with the primers were scored as 1 for presence or 0 for absence. To measure the informativeness of the markersto differentiate between genotypes, polymorphism information content (PIC), Effective multiplex ratio (EMR), marker index (MI) and resolving power (RP) were calculated. PIC was calculated according to the formula of (Anderson *et al*., 1993), as follow:

$$PIC=1-\Sigma pi^2$$

where pi is the frequency of the *i*th allele of the locus in eight genotypes. Effective multiplex ratio (EMR) is calculated as total number of polymorphic loci (per primer) multiplied by the proportion of polymorphic loci per their total number (Powell *et al*., 1996; Nagaraju *et al*., 2001):

$$EMR = np\ (np/n)$$

where np is the number of polymorphic loci, and n is the total loci number. Marker index (MI) is the product of the polymorphism information content value and effective multiplex ratio (Powell *et al*., 1996; Nagaraju *et al*., 2001):

$$MI = PIC \times EMR.$$

The Rp of each primer was calculated using the formula: $Rp = \Sigma\ Ib$,

where Ib is band informativeness (the Ib can be represented on a scale of 0–1 by the following formula:

$$Ib = 1-(2 \times \left| 0.5 - p \right|)$$

Where, p is the proportion of individual containing the band (Prevost and Wilkinson, 1999).

RESULTS AND DISCUSSION

Tomato is the most important fruit crop in the world, as well as in Egypt. A total of eight genotypes of the cultivated tomato are studied. Molecular markers, along qualitative and quantitative morphology characters represent a resilient and rapid tool for characterizing diversity within the target species. Variable efficiencies of different marker systems for detecting DNA polymorphism in tomato have been reported RAPD (El-Hady *et al*., 2010; Mansour *et al*., 2010; Naz *et al*., 2013), SSR (El-Awady *et al*., 2012; Singh *et al*., 2014), ISSR (Mansour *et al*., 2010; Aguilera *et al*., 2011; Shahlaei *et al*., 2014) and SCoT (Shahlaei *et al*., 2014).Two of these marker systems, SCoT and ISSR, were employed in the present study for detecting genetic diversity and relationships among eight genotypes of *Lycopersicum esculentum* L. Our results indicated that primers, successfully amplified accessions template DNAs. Salient features of fingerprint database obtained using different markers are given below.

Polymorphism as detected by ISSR Analysis

Six ISSR primers (Table 3) were used to study the genetic diversity and relationships among the eight tomato genotypes. These primers produced multiple band profiles (Fig. 1). Table (3) showed different genetic diversity parameters studied for ISSR primers. These primers revealed a distinct scorable fragment per primer and in total, 55 bands, both polymorphic and monomorphic were obtained. The overall size of amplified products ranged from 130 to 4010 bp. Out of 55 bands, 26 bands were polymorphic and 29 bands were monomorphic. Maximum number of polymorphic bands (8 of 14 bands) were obtained for HB-15 primer where the minimum number of polymorphic bands (1 of 9 bands) were obtained for HB-12 primer.In the same context, (Henareh *et al*., 2016) studied genetic variability by using 20 ISSR primers, 14 primers amplified 185 scorable loci, which all were polymorphic (100%). The number of total loci per primer ranged from 6 to 26, with an average of 13.2. In addition, (Sharifova *et al*., 2017) found that in total, 50 scorable bands were obtained from studied tomato accessions, where 18 out of them were monomorphic, occurring in all representatives, and 32 were polymorphic, representing 63.3% of all the amplified loci.

The polymorphism percentage averaged to 47.27% across all the genotypes. The highest percentage of polymorphic was (60%) for primer 14A and the lowest was (11.1%) for primer HB-12. In the same context, high level of polymorphism in tomato cultivars was (100%) founded by Mansour *et al*. (2010) and Henareh *et al*. (2016). And was 62% founded by (Edris *et al.,* 2014). Contrary,

low level of polymorphism (Aguilera *et al*., 2011) (34%) and (Shahlaei *et al*., 2014) (23.25%). In addition, (Sharifova *et al*., 2017) showed that level of polymorphism ranged from 50 to 90% and ISSR primer (UBC825 and HB8) were the most (10) and the less (3) allele produced polymorphism.

The polymorphism information content (PIC) analysis was carried out to determine the efficiency of each ISSR marker to express polymorphic loci in tomato (Table 3). The PIC index has been used extensively in many genetic diversity studies (Tatikonda *et al*., 2009; Thudi*et al*., 2010). Moreover, PIC has been used usually for evaluating the informative potential of ISSR markers in different germplasm and cultivated genotypes (Grativol *et al*., 2011). The calculated PIC values for ISSR markers ranged from 0.11 to 0.50. The highest mean PIC value of 0.50 expressed by primer HB-15 indicated that this primer was the most polymorphic.The primer HB-12, which yielded the lowest mean PIC value of 0.11, was the least polymorphic. Our result of PIC values was differed from Shahlaei *et al*. (2014) whereas average was 0.088. Meanwhile, Henareh *et al*. (2016) founded that the mean of heterozygosis for the primers varied from 0.153 to 0.30.

Marker index (MI) is a feature of a marker and was calculated for all the primers. The MI values ranged between 0.01 and 2.29 with average 1.03. The maximum MI (2.29) was observed for the primer HB-15 and the minimum MI (0.01) was obtained with ISSR primersHB-12. In addition, EMR is the product of the fraction of polymorphic bands and the number of polymorphic bands and therefore the higher polymorphism provides higher EMR. EMR values varied from0.11 to 4.57 for primers HB-12 and HB-15, respectively, whereas the mean value was 2.33. These two features have been used to evaluate the discriminatory power of molecular marker systems in some plant species e.g. apricot (ISSR, EMR = 4.8, MI = 3.74) (Kumar *et al*., 2009), Jatropha (AFLP, EMR = 97, MI = 25.13) (Tatikonda *et al*., 2009), Pongamia (AFLP, EMR = 77.2, MI = 16.83) (Thudi *et al*., 2010).

Resolving power (RP) is a parameterused to characterize the ability of the primer/marker combination to detectthe differences between many genotypes (Prevost and Wilkinson, 1999). RP values of the six ISSR primers varied from 8 to 16 distinguishing the different genotypes while the average was 12.50 per ISSR primer. The highest RP values were observed with the ISSR primer HB-12 & HB-15 (16) and the lowest with the ISSR primer HB-9 (8) (Table 3). In this regard, Shahlaei *et al*. (2014) found that the mean of (RP) values for ISSR markers was 1.55.

In general, our results showed that primer HB-15 and HB-12 had highest and lowest values for (P%, PIC, MI and EMR), respectively and interestingly for RP value both primers were the highest values. The results indicated that ISSR

markers could be used effectively to analyze the genetic diversity in tomato. There are several a published report analysis of genetic diversity of tomato varieties at the varietal and species levels, which was determined successfully by ISSR markers (Terzopoulos and Bebeli, 2008; Aguilera *et al*., 2011).

Polymorphism as detected by SCoT analysis

Seven SCoT primers were used tostudy the genetic differences and relationships among the eight tomato genotypes asshown in (Fig. 2) and (Table 3). A total of 73 bands were generated.The total bands per primer ranged from 5 (SCoT 6) to18 (SCoT 10). Out of 73 bands, 44 bands were polymorphic and 29 bands were monomorphic. The overall size of amplified products ranged from 200 to 2320 bp. Maximum number of polymorphic bands (14 of 18 bands) were obtained for SCoT 10 primer whereas the minimum number of polymorphic bands (1 of 9 bands) were obtained for SCoT 11 primer. The polymorphism percentage averaged to 0.60 across all accessions. The highest percentage of polymorphic was (80%) for primer SCoT9 and the lowest was (11.11%) for primer SCoT11. These results agree with those of (Shahlaei *et al*., 2014) who used SCoT for genetic diversity analysis of 10 tomato accessions. Using ten selected SCoT primers 83 bands were generated, of which 30 (36.14%) were polymorphic.

PIC values varied from 0.10 (SCoT 11) to 0.64 (SCoT 9) with an average of 2.9. Similarly, Shahlaei *et al*. (2014) results showed that PIC average value for SCoT was 0.142. Our results indicated that these loci were highly informative and demonstrating uniform polymorphism rate among all the seven SCoT primers.

Moreover, the average of MI value was 2.27. Meanwhile, the highest and lowest values of MI were observed for SCoT 10 (6.42) and SCoT 11 (0.01), respectively. In addition, EMR values ranged from 0.11 to 10.89 for primers SCoT 11 and SCoT 10, respectively and the mean value was 4.28.

Resolving power parameter is described by (Prevost and Wilkinson, 1999) as a degree of the discriminatory power of molecular markers. The RP value of the seven primers varied between 9 (SCoT 6) to 20 (SCoT 10) discriminating the different genotypes. While the average RP was 14.14 (Table 3). In this regard, Shahlaei *et al*. (2014) found that the mean of RP value for SCoT was 1.88.

To sum up, ourresults showed that primers SCoT 9 and SCoT 10 had highest values for (P%, PIC, MI and EMR) whereas the lowest value was for primer SCoT 11. Moreover, for RP the highest values were for primers SCoT 10 and SCoT 11 and the lowest value was for primer SCoT 6. The results also demonstrate that the SCoT marker system is more informative than ISSR for identification and genetic diversity analysis of tomato genotypes. These results were in harmony with Shahlaei *et al*. (2014).

More importantly, SCoT marker is generated from the functional region of the genome; the genetic analyses using this marker would be more useful for crop improvement programs, such as genotype identification, considering genetic diversity, construction of linkage maps and QTL mapping.

Comparison of SCoT and SSR data

From one hand, ISSR and SCoT markers have been proved to be useful in genetic diversity studies because of their high reproducibility and great power for the detection of polymorphism (Guo *et al*., 2012; Hamidi *et al*., 2014). In the present study, our results showed that there is a high genetic variation among tested genotypes, which agrees with the results from previous studies carried out on tomato. The efficiency of both ISSR and SCoT markers is estimated through parameters such as (P%, PIC, MI, EMR and RP).

PIC refers to the values of a marker for detecting polymorphism within a population or set of genotypes by considering not only the number of alleles that are expressed but also the relative frequencies of alleles per locus. Based on the results, the moderate and high values of PIC (averaged of 0.37 and 0.41) for ISSR and SCoT markers, respectively, could be attributed to evaluation of genetic diversity. The MI & EMR which can be a common measure of efficiency in discovering polymorphism (Khodadadi *et al*., 2011) and considered important property of a suitable marker system is the capacity to distinguish among different accessions. Our results indicate that SCoT marker was higher than ISSR for these parameters. In addition, our study revealed that the resolving power of SCoT (averaged 14.14) primers is higher than ISSR primers. Consequently, these results agree with previous studies (Prevost and Wilkinson, 1999; Khodadadi *et al*., 2011).

From other hand, all eight tomato genotypes were characterized by 24 genotype-specific markers asshown in Table (4). These marker loci were classified as 21 positive and 3 negative. By ISSR primers, there are 12 genotype-specific markers (all are positive markers). Meanwhile by SCoT primers, there are 12 genotype-specific markers (nine positive markers and three negative markers). Moreover, we concluded that the primer SCoT 10 reveled four genotype-specific markers (three positive and one negative) while primer HB-15 reveled 4 positive genotype-specific markers. Among the studied genotypes, we found that the highest genotype had genotype-specific markers were genotype Super Queen showed 10 positive genotype specific markers (eight markers of them revealed by ISSR and the others two markers revealed by SCoT); then genotype Hellfrucht showed six genotype specific markers (five positives and one negative) and all are revealed by SCoT.Then coming the rest of genotypes as follow (G10340 and Hildares) had three and two genotype-specific markers, respectively, and (Agyad 7, Agyad 16 and Falcon) had one genotype-specific markers. And just

genotype Yayla has not any specific markers.

These results indicated that all types applied in thisstudy succeeded in showing different molecularmarker patterns which can be relied upon in distinguishing among thestudied tomato genotypes. In general, SCoT marker type had the highest percentage than ISSR ofall studied parameters (P%, PIC, MI, EMR and RP).These findings were in harmony withthat illustrated previously by (Shahlaei *et al*., 2014) in tomato.

Phylogenic relationship as detected by genetic similarity (GS) usingSCoT and ISSR data

Table (5), showed the phylogenic relationship reveled from genetic similarity (GS) using ISSR data. The ISSR in this study showed a genetic similarity ranged from 0% to 100%.The highest genetic similarity was revealed by the ISSR analysis was 100% between genotype Super Queen and genotype G10340, followed by 97% between genotype Super Queen and genotype Hellfrucht. This can be explained by narrow genetic differences between those genotypes. On the other hand, the lowest genetic similarity was 0% between genotype Hellfrucht and genotype Falcon followed by 19% between genotype Hildares and genotype Hellfrucht and between genotype Agyad 16 and genotype Yayla. In this regard, Terzopoulos and Bebeli (2008) and Sharifova *et al*. (2017) found that the similarity coefficient based on ISSR markers ranged from 0.56 to 0.95 and ranged from 0.52 to 0.98, respectively.

Moreover, Table (6) showed the phylogenic relationship revealed from genetic similarity (GS) using SCoT data. As ISSR marker SCoT product showed a genetic similarity ranged from 0% to 100%. The highest genetic similarity revealed by the SCoT analysis was 100% among genotype Super Queen and genotype Falcon, followed by 89% between genotype Super Queen and genotype Hellfrucht. On the other hand, the lowest genetic similarity was 0% between genotype Super Queen and genotype Agyad 7 followed by 9% between genotype Hildares and genotype Falcon.

The ISSR data have confirmed The SCoT data, when combined both data together. The similarity matrix resulting from the combined DNA markers SCoT and ISSR data were performed to generate correct relationships based on large and different genome regions as shown in (Table 7). The highest percentage of genetic similarity (100%) was detected between genotype Super Queen and genotype Falcon, followed by 95% between genotype Super Queen and genotype Hellfrucht, indicating that these two tomato genotypes are closely related to each other's. On the other hand, the lowest genetic similarity value (0%) between genotype Agyad 7 and genotype Agyad 16 followed by 1% between genotype Hildares and genotype Falcon indicating the wide genetic diversity among them. These results confirmed the result ob-

tained by ISSR analysis published by Abu Qamar *et al*. (2008), indicating the wide genetic diversity among them.

Phylogenic relationship as detected by cluster analysis using SCoT and ISSR

To investigate, genetic relationships among studied genotypes cluster analysis based on Jaccard's similarity coefficients and UPGMA algorithm were calculated for the eight tomato genotypes. According to the clustering pattern obtained by ISSR (Fig. 3) data. The eighttomato genotypes classified into five groups, based on ISSR dendrogram, the first group consists of genotype Hellfrucht along with genotype Falcon. Two genotypes Agyad 16 and Yayla were classified in a separate group (the second group). The third group consists of two genotypes Super Queen and Agyad 7. Finally, fourth and fifth groups consist of one genotype each Hildares and G10340, respectively. In the same context, (Terzopoulos and Bebeli, 2008; Sharifova *et al*., 2017; Henareh *et al*., 2016) studied phylogenic relationship by cluster analysis using the UPGMA method and placed all studied tomato genotypes into 2, 6 and 9 clusters respectively. Also, Mansour *et al*. (2010) found that different dendrograms constructed for the RAPD, ISSR and IRAP results individually and collectively reveal that similarity and clustering are highly dependent on the marker system used.

According to SCoT dendrogram (Fig. 4), two tomato genotypes were placed in the first group Super Queen and Agyad 7. Also, the second group consists of two genotypes Hildares and Falcon. Another two genotypes Yayla and Hellfrucht were clustered in the third group. The rest of genotypes along with genotype Agyad 16 were placed into the fourth group and genotype G10340 was also separated as the fifth group.

The dendrogram built based on combined data from, SCoT and ISSR analyses showedin (Fig. 5), and represents the genetic similarity among the eight tomato genotypes. The dendrogram includes six clusters; the first group contained genotypes Agyad 7 and Agyad 16. While the second group include genotypes Hildares and Falcon seems for SCoT dendrogram. In addition, in this dendrogram represent four groups includes just one genotype Super Queen, Yayla, Hellfrucht and G10340. In another study, SCoT and ISSR were used for study the genetic diversity forten genotypes and were clustered into three major groups based on the SCoT analysis and two major groups based on the ISSR analysis with UPGMA (Shahlaei *et al*., 2014). Good results could be obtained if we crossed these eight genotypes because there is a wide diversity among them. It is noteworthy that cluster analysis is a valuable tool for subdividing genotypes into groups including similar and dissimilar lines and has a great value from the breeder's point of view for initiating tomato breeding program. These findings are in line with those obtained earlier by (Svobodova *et al*., 2011; Maniruzzaman, 2014).

The finally, results of this study demonstrated that the existence of high genetic variation in the tomato studied genotypes. This genetic variation can be explored in tomato breeding programs for hybrid cultivars production. Moreover, this study considered as the starting point needed to identify the valuabletomato genotypes germplasm at phenotype levels and draw the attention of breeders towards this valuable yet germplasm. This is especially significant since molecular analysis has proved to be a promising strategy in the selection of germplasm.

Genetic variability and relationships among eight tomato genotypes were evaluated by using six ISSR primers and seven SCoT primers. A high degree of polymorphism was detected with the two types of DNA markers which recorded 47.21% and 60.30%, respectively. Allele's number ranged from7 to 14 and 5 to 18 per primer, with averages of 9.17 and 10.43 per ISSR and SCoT primers, respectively. The highest percentage of genetic similarity as revealed by combined SCoT and ISSR data was found between genotype Super Queen and Falcon (100%), while the lowest similarity percentage was detected between genotype Agyad 7 and genotype Agyad 16 (0%). HB-15 and HB-12 had highest and lowest values for studied parameters, respectively and interestingly for RP value both primers were the highest values. Moreover, primers SCoT 9 and SCoT 10 had highest values for all studied parameters whereas the lowest values were for primer SCoT 11 except for RP value.

Our results found 24 genotype-specific markers (21 positive and 3 negative). Twelve from ISSR (all are positive markers), twelve from SCoT (9 positive and 3 negative markers). Primer SCoT10 revealed 4 genotype-specific markers (3 positives and 1 negative) while primer HB-15 reveled 4 positive genotype-specific markers. Genotype Super Queen had the highest genotype-specific markers (10 positive markers) 8 markers were by ISSR and 2 markers were by SCoT; then genotype Hellfrucht showed 6 genotype specific markers (5 positive and 1 negative) and all by SCoT. Therefore, these genotype-specific markers could be considered as a molecular marker for those genotypes.

In general, ISSR and SCoT markers system is useful for identification and genetic diversity analysis of tomato genotypes but in the same time SCoT marker type had the highest percentage than ISSR of all studied parameters and more informative than ISSR.

SUMMARY

Two molecular marker systems, SCoT and ISSR were used for genetic diversity analysis of eight tomato (*Lycopersicum esculentum* L.) genotypes. The molecular markers revealed robust amplification profiles. Using seven selected SCoT primers 63 bands were generated, of which 38 (60.3%) were polymorphic. Six selected ISSR primers amplified 55 bands with 26 (47.3%) being polymorphic. (PIC), (EMR), (MI) and (Rp) of the primers were calculated for

thetwo marker systems and all the parameters examined found to be higher in SCoT system. SCoT and ISSR revealed different genetic similarity among the eight tomato genotypes. SCoT and ISSR techniques characterized the studied genotypes by many unique markers throughout 12 unique markers, for every technique. Genotype Super Queen showed 10 positive genotype specific markers; followed by genotype Hellfrucht showed six genotype specific markers (five positives and one negative) indicating that these markers may be associated with a feature that has not yet been determined. Consequently, SCoT markers would be a better choice compared to ISSR markers in characterization of tomato genotypes. Also, it can be concluded that in future study of genetic diversity like heremore than one marker systems should beused for higher genetic resolution of the genome.

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Table (1): Genotypes code, genetic resources and names of eight tomato genotypes.

Genotypes Number	Genotypes Code	Origin	Name
1	TM SR1	SYRIA	SUPER QUEEN
2	TM HB 1	EGYPT	AGYAD 7
3	TM HB 2	EGYPT	AGYAD 16
4	TM TK 1	TURKI	YAYLA
5	TM GR 1	GERMANY	HILDARES
6	TM GR 2	GERMANY	HELLFRUCHT
7	TM TK 2	TURKI	FALCON
8	TM GR 3	GERMANY	G10340

Table (2): List of the primer names and their nucleotide sequences used in the study for ISSR and SCoT procedure.

ISSRprimers			SCoTprimers		
No.	Name	Sequence	No.	Name	Sequence
1	14A	CTC TCT CTC TCT CTC TTG	1	SCoT 1	ACG ACA TGG CGA CCA CGC
2	44B	CTC TCT CTC TCT CTC TAG	2	SCoT 3	ACG ACA TGG CGA CCC ACA
3	HB-09	GTG TGT GTG TGT GC	3	SCoT 6	CAA TGG CTA CCA CTA CAG
4	HB-12	CAC CACCAC GC	4	SCoT 8	ACA ATG GCT ACC ACT GAG
5	HB- 14	CTC CTCCTC GC	5	SCoT 9	ACA ATG GCT ACC ACT GCC
6	HB-15	GTG GTGGTG GC	6	SCoT10	ACA ATG CTA CCA CCA AGC
			7	SCoT 11	ACA ATG GCT ACC ACT ACC

Table (3): Number and types of the amplified DNA bands as well as the polymorphism percentage generated by the six ISSR and seven SCoT primers for eight tomato genotypes.

Marker	Name of markers	Number of fragments				FS bp						
		MB	UB	PB (with Unique band)	TAB	Larger	Smaller	PIC	EMR	MI	P (%)	RP
ISSR	14A	4.00	1.00	6.00	10.00	1550	130	0.40	3.60	1.44	60.00%	14.00
	44B	3.00	1.00	4.00	7.00	1480	270	0.44	2.29	1.01	57.14%	9.00
	HB-09	3.00	3.00	4.00	7.00	1070	295	0.48	2.29	1.10	57.14%	8.00
	HB-12	8.00	1.00	1.00	9.00	2355	320	0.11	0.11	0.01	11.11%	16.00
	HB-14	5.00	2.00	3.00	8.00	390	230	0.30	1.13	0.34	37.50%	12.00
	HB-15	6.00	4.00	8.00	14.00	4010	230	0.50	4.57	2.29	57.14%	16.00
	Total	29.00	12.00	26.00	55.00			2.23	13.98	6.18	47.27%	75.00
	Average	4.83	2.00	4.33	9.17			0.37	2.33	1.03	46.65%	12.50
SCoT	Scot1	4.00	3.00	6.00	10.00	900	235	0.51	3.60	1.84	60.00%	12.00
	Scot 3	3.00	1.00	7.00	10.00	1565	235	0.45	4.90	2.25	70.00%	13.00
	Scot 6	2.00	0.00	3.00	5.00	590	265	0.26	1.80	0.47	60.00%	9.00
	Scot 8	6.00	1.00	5.00	11.00	2320	240	0.34	2.27	0.77	45.45%	17.00
	Scot 9	2.00	1.00	8.00	10.00	1185	270	0.64	6.40	4.10	80.00%	11.00
	Scot 10	4.00	3.00	14.00	18.00	1640	200	0.59	10.89	6.42	77.78%	20.00
	Scot 11	8.00	0.00	1.00	9.00	740	220	0.10	0.11	0.01	11.11%	17.00
	Total	29.00	9.00	44.00	73.00			2.90	29.97	15.86	60.27%	99.00
	Average	4.14	1.29	6.29	10.43			0.41	4.28	2.27	57.77%	14.14

MB: monomorphic band, UB: unique band, PB: polymorphic band, TAB: total amplified bands, FS: fragment size, PIC: polymorphic information content, EMR: effective multiplex ratio, MI: marker index, P%: percent of polymorphism and RP: resolving power.

Table (4): Positive and negative genotype-specific markers and their molecular sizes (bp) and total number of markers for each genotype using ISSR and SCoT analysis.

Positive marker				
Marker	Genotype	primer	size	No.
ISSR	Super Queen	14A	400	1
	G10340	44B	613	1
	Super Queen	HB-09	1070	3
	Agyad 7		815	
	Super Queen		636	
	Super Queen	HB-12	2357	1
	Super Queen	HB-14	895	2
	Hildares		5602	
	Super Queen	HB-15	4012	4
	Super Queen		2869	
	Super Queen		2175	
	G10340		1474	
SCoT	Hellfrucht	Scot1	897	3
	Hellfrucht		845	
	Hellfrucht		746	
	Hellfrucht	Scot 3	1564	1
	Hellfrucht	Scot 8	1832	1
	Super Queen	Scot 9	1020	1
	Agyad 16	Scot 10	665	3
	G10340		625	
	Super Queen		960	
Total				21
Negative marker				
SCoT	Falcon	Scot 3	430	1
	Hellfrucht	Scot 6	491	1
	Hildares	Scot 10	1147	1
Total				3

Table (5): Similarity index of ISSR analysis of eight tomato genotypes.

	1	2	3	4	5	6	7
2	0.54						
3	0.74	0.31					
4	0.81	0.52	0.19				
5	0.91	0.54	0.32	0.34			
6	0.97	0.63	0.43	0.42	0.19		
7	0.94	0.58	0.38	0.37	0.26	0.00	
8	1.00	0.74	0.55	0.43	0.44	0.43	0.38

Table (6): Similarity index of SCoT analysis of eight tomato genotypes.

	1	2	3	4	5	6	7
2	0.00						
3	0.13	0.20					
4	0.34	0.34	0.37				
5	0.74	0.74	0.78	0.25			
6	0.89	0.81	0.68	0.29	0.28		
7	1.00	0.72	0.77	0.53	0.09	0.55	
8	0.53	0.43	0.46	0.34	0.45	0.37	0.42

Table (7): Similarity index of SCoT and ISSR combination analysis of eight tomato genotypes.

	1	2	3	4	5	6	7
1							
2	0.13						
3	0.36	0.00					
4	0.52	0.33	0.16				
5	0.82	0.59	0.50	0.22			
6	0.95	0.70	0.50	0.23	0.18		
7	1.00	0.61	0.51	0.36	0.01	0.18	
8	0.75	0.52	0.43	0.27	0.35	0.29	0.29

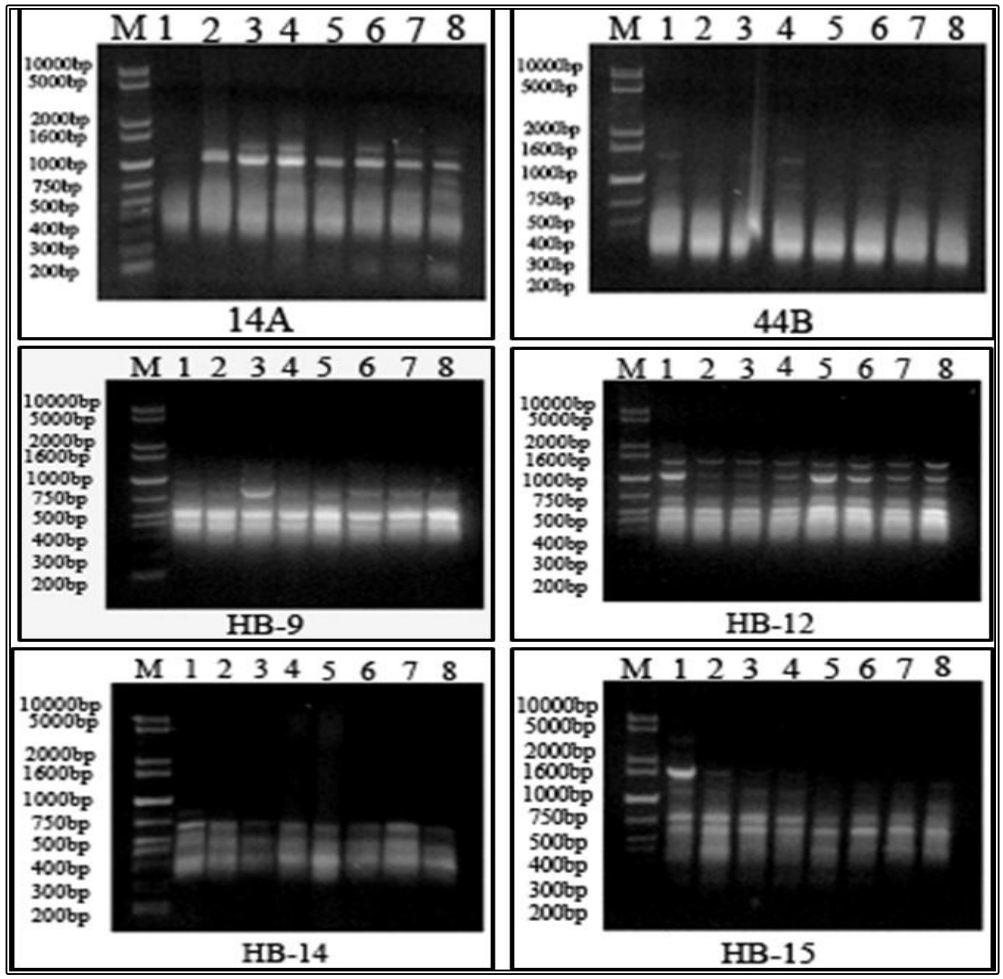

Fig. (1): ISSR patterns of the eight tomato genotypes revealed by six primers.

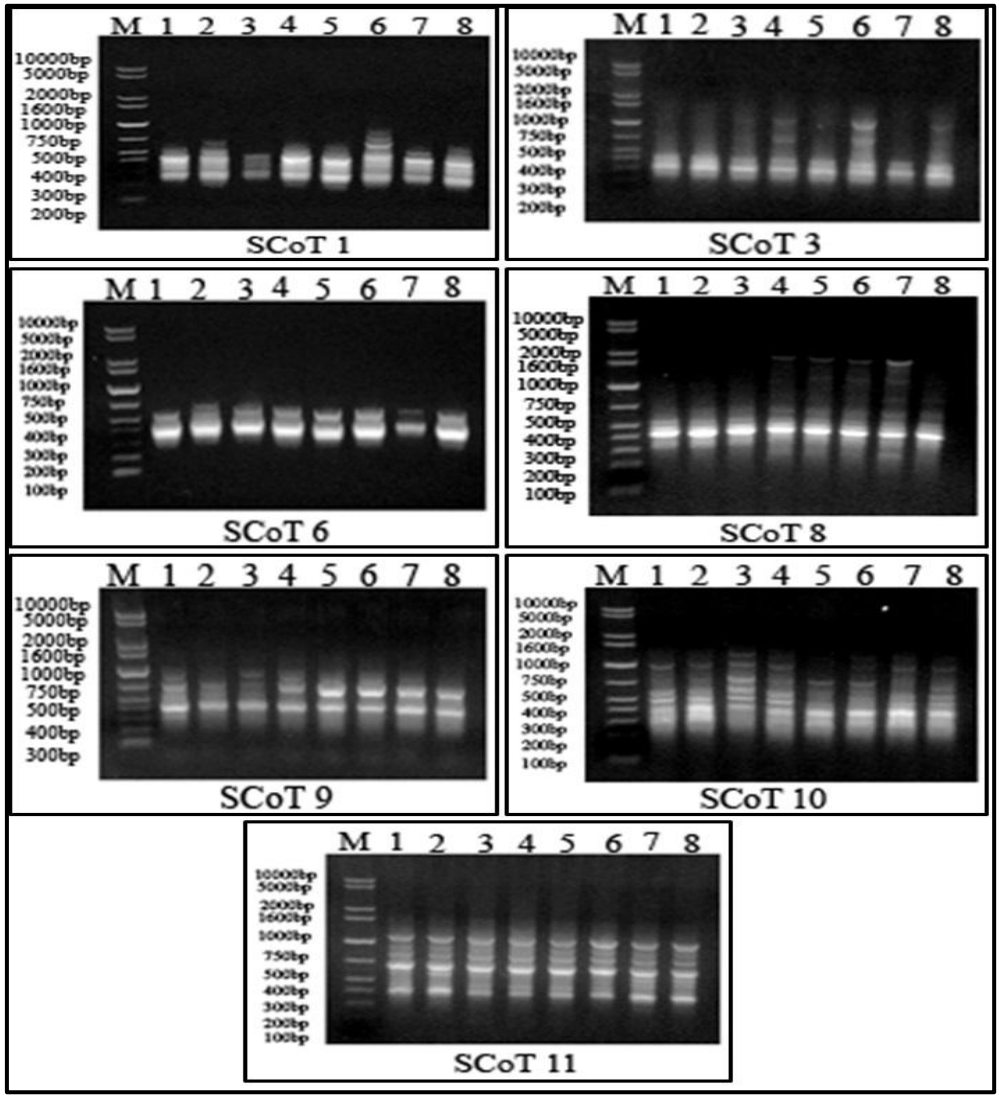

Fig. (2): SCoT patterns of the eight tomato genotypes revealed by six primers.

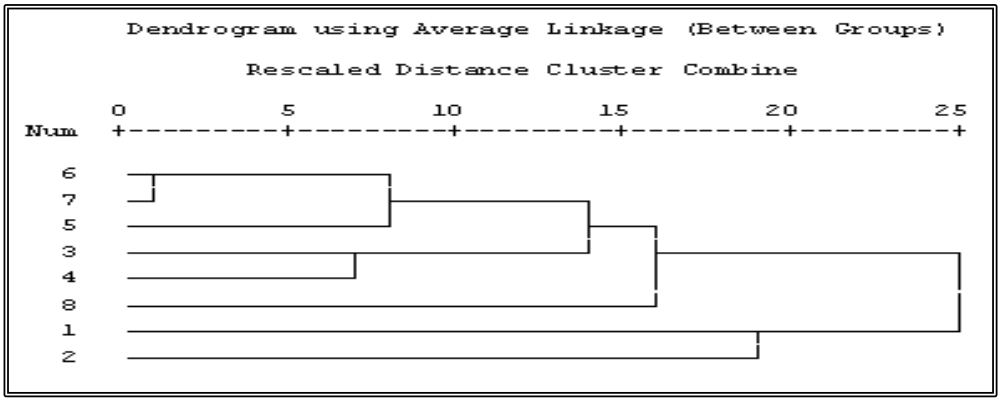

Fig. (3): Dendrogram of ISSR analysis of eight tomato genotypes.

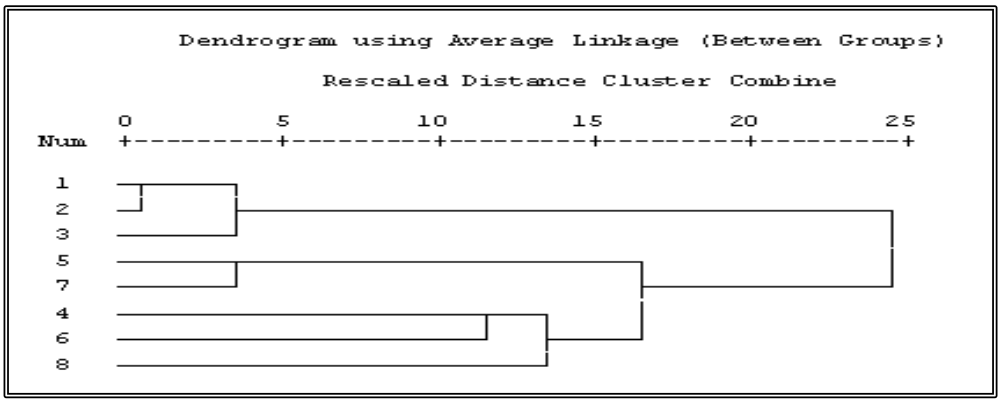

Fig. (4): Dendrogram of SCoT analysis of eight tomato genotypes.

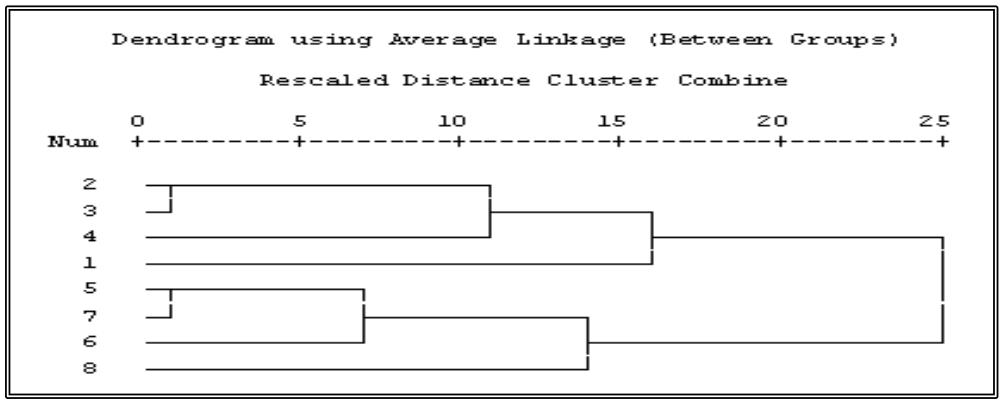

Fig. (5): Dendrogram of SCoT and ISSR combination analysis of eight tomato genotypes.