Genetic Diversity of Some Tomato *Lycopersicon esculentum* Mill Varieties in Iraq Using Simple Sequence Repeat (SSR) Markers

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Abstract

This study was conducted to evaluate the genetic diversity among 19 tomato varieties (determinate and indeterminate) cultivated in Iraq using polymerase chain reaction based DNA marker (PCR based DNA markers Simple Sequence Repeats (SSRs). To achieve PCR reactions, total genomic DNA was isolated from fresh leaves (2 weeks old). The average yields of DNA were in the range of 100-295 ng/μl with a purity ranging between 1.8-1.9.

A total of 21 alleles were detected among the tested varieties using 5 SSRs loci distributed on 4 chromosomes of tomato. The molecular size of bands obtained from amplification of SSR products ranged from 121 to 247 bp. Alleles ranged from one in (Tom 8-9, Tom 41-42 and Tom 67-68 loci) to twelve in Tom 49-50 locus. The values of heterozygosity for each locus ranged between 0.63 for Tom 31-32 and 0.89 for Tom 49-50 with a mean value of 0.30. The polymorphic information content (PIC) values for the SSR loci ranged from 0.45 in Tom 31-32 to 0.58 in Tom 49-50 loci with an average of 0.21. Each one of (Tom 8-9, Tom 41-42 and Tom 67-68 loci) produce 0.0 value for both heterozygosity and PIC.

The study revealed that, The lowest genetic distance was (0.3244) between varieties Kenanh (lane5) and Warda (lane10), while, the highest genetic distance was (0.9177) between varieties Helam (lane 3) and Super marimond (lane 16). The genetic similarity values ranging from (0.0823 to 0.6756) depending upon the genetic distance values that ranging from (0.3244 to 0.9177), indicating the largest diversity with percentage of (32 to 91%) among the tested varieties.

The analysis of the obtained results from genetic distances and Neighbor-joining dendrogram (unrooted tree) revealed that, the 19 tested tomato varieties can be grouped into two major groups: first cluster include 9 varieties distributed in two subgroups. The second major cluster include 10 varieties which in turn divided into two subgroups.
Key words: Genetic diversity; *Lycopersicon esculentum*; SSR markers; genetic distance.

Introduction

Tomato (*Lycopersicon esculentum*) is one of the most important vegetable crop grown throughout the world; it is second important vegetable after potato because of its wider adaptability, high yielding potential and multipurpose uses (37). It is a member of the family Solanaceae and significant vegetable crop of special economic importance in the horticultural industry worldwide (16;44).

In addition to its economic importance, tomato has proven to be a useful model in the study of plant genetics, molecular biology, and chromosome structure (91), moreover, tomato considered a model research organism because it has a relatively short life cycle, (8) and ease of culture under a wide range of environments (1; 29).

An improvement in yield and quality in self-pollinated crops like tomato is normally achieved by selecting the genotypes with desirable character combinations existing in nature or by hybridization. The success of hybridization programme depends upon selection of suitable parents of diverse origin (37).

Microsatellite known as simple Sequence Repeats (SSRs), are sections of DNA consisting of tandemly repeating mono-, di-, tri-, tetra-, penta- and hexa-nucleotide units that are arranged throughout the genomes of most eukaryotic species (30). In addition to its high level of polymorphism, this technique is useful molecular markers because they are abundant, uniformly distributed, codominant, rapidly produced by PCR, relatively simple to interpret and easily accessed by other laboratories via published primer sequences. Besides, for measuring diversity, it is very useful tool for assigning lines to heterotic groups and for genetic fingerprinting (28; 32).

SSR markers are of great importance for the genetic diversity and variability for tomato variety (6;16 and 14). There is a need to extensively collect, exploit and evaluate existing tomato germplasm for breeding suitable varieties. As improvement of the tomato crop would enhance agricultural productivity and facilitate food security (13), furthermore, characterization of varieties and hybrids which are of wider acceptance by farming community need to be studied in order to regulate their genetic purity during their multiplication and seed quality evaluation (43).

Materials and Methods


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Samples Collection:

A collection of tomato varieties with different growth habit (determinate and indeterminate) samples of tomato leaves (2 weeks old) were collected for DNA isolation.

DNA Isolation:

The Genomic DNA Mini Kit (Geneaid Biotech. Ltd; Taiwan Company). The PCR reaction mixture was prepared as follows: 1. 5µl template DNA and 4 µl of primer (10 pmole/µl, 2µl forward and 2µl reverse), were added to each AccuPower® TLA PCR PreMix tube. 2. Sterilized deionized distilled water was added to AccuPower® TLA PCR PreMix tubes to the final volume of 20 µl. 3. The tubes were mixed with vortex to dissolve the lyophilized blue pellet, and briefly spine down (all these steps were done in ice). a sequence was amplified individually using SSR primer (listed in table 1).

Amplification were performed in thermocycler programmed according to annealing temperatures as follows: one cycle of 3 min at 94°C, for 37 cycle of each 100 sec at 94°C, 100 sec at 56°C and 2 min at 72°C, with a final extension for one cycle of 10 min at 72°C (Tom322-323, Tom 8-9, Tom 31-32, Tom 41-42, Tom 49-50, Tom 61-62, Tom 67-68).

Table 1: Names and sequences of SSR primers used in this study.

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<tr>
<th>Primer</th>
<th>Sequences (5’ → 3’)</th>
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<td>Tom322-323</td>
<td>Forward: GGTGAAAAGAGCAAAATAGT&lt;br&gt;Reverse: TTTGTAATCCATGTCTTATAA</td>
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<td>Tom 8-9</td>
<td>Forward: GCATTGATTGAACTTCATTCTCGTCC&lt;br&gt;Reverse: ATTTTTGTCCACCAA ACTAACCG</td>
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<td>Tom 31-32</td>
<td>Forward: AATGTCTTTCGTATCCTTTCGT&lt;br&gt;Reverse: CTCGGTTTTATAATTTTG TGTCT</td>
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<td>Tom 41-42</td>
<td>Forward: GAAATCTGTGGAAGCCCTCTC&lt;br&gt;Reverse: GAC TGTGATAGTAAGAATGAG</td>
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<td>Tom 61-62</td>
<td>Forward:GGCAAAGAAGGACCAGGC&lt;br&gt;Reverse: GGTGCCTAAAAGATGAAAT</td>
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<td>Tom 49-50</td>
<td>Forward: AAGAAACTTTTTGGATGTGTC&lt;br&gt;Reverse: ATTACAATTTGAGAGTGCAAGG</td>
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<td>Tom 67-68</td>
<td>Forward: TCCACACCCCTACACC AT&lt;br&gt;Reverse: TAACACGTCACACAAGGAC</td>
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</table>
Then amplified DNA product were separated by electrophoresis using MetaPhor® agarose gel (the high resolution agarose that challenges polyacrylamide) (2%) 2% (1.5-2 hr, 70 V) to be sure that the PCR process succeeded (47). PCR products were visualized by U.V transilluminator and then were imaged by gel documentation system (15), the size of SSR-PCR products estimated by comparing with the marker 100 bp DNA ladder 100-2,000 bp.

Data Analysis of SSR Products:

The resulting microsatellite data were analysed using Power Marker V.3 software (http://www.powermarker.net) to calculate the number of alleles, heterozygosity and polymorphic information content (PIC). Genetic distance was computed using(27) standard genetic distance (Ds). The distance method of (27) was used with program Power Marker V.3 for the construction of phylogenetic tree. Neighbore-joining method was used to obtain the tree. The tree was then viewed using the TREEVIEW version 1.6 (http://taxonomy.zoology.gla.ac.uk/rod/rod.html) programs.

Results and Discussion

Seven microsatellite markers (Tom 8-9 ,Tom 31-32,Tom 41-42 ,Tom 49-50,Tom67-68, Tom322-323 and Tom 61-62) were used to test gene diversity of nineteen tomato varieties Table 1. Two of them (Tom322-323 and Tom 61-62 ) failed to amplify the expected PCR fragment.

When SSR-PCR products were separated by agarose gel electrophoresis (Figures1and 2) either one band (homozygous) or two bands (heterozygous) were noticed for each primer depending on the types of microsatellite loci (homozygous or heterozygous) (8). Microsatellites as they are co-dominant marker thus heterozygote produces two bands revealing the amplification of the two loci and could be readily identified (35; 47).SSR increases the efficiency and accuracy of population genetic measures based on these markers compared with other markers (Wang etal.,2009).

Data in table 2 show that the molecular size of these bands obtained from amplification of SSR products of nineteen investigated tomato varieties ranged from 121 bp to 247 bp. Another study reported that molecular size could range from 75 bp to 285 bp in differ collection of ten tomato cultivars in Egypt using twenty SSR markers some of them involved in this study(10).
The total number of alleles detected was 21 for five SSR loci in nineteen tomato varieties. The alleles ranged from one in (Tom8-9, Tom41-42 and Tom67-68) to twelve in Tom49-50 loci. The allele number was higher than that obtained in combination of sixty-three tomato varieties in Korea using thirty three SSR marker in which higher allele number reaches to seven (19). Another investigations between several tomato cultivar in Spain, Argentina in which allele number could reach nine or three as maximum value (32 and 49).

### Table 2: Summary of genetic diversity at five SSR loci in tomato. Primer name, no. chromosome, repeat type, fragment size range (bp), no. of amplified bands, number of alleles, heterozygosity and polymorphic information content (PIC).

<table>
<thead>
<tr>
<th>No.</th>
<th>Primer</th>
<th>No. chromosome</th>
<th>Repeat type</th>
<th>Fragment size range (bp)</th>
<th>No. of amplified bands</th>
<th>No. of alleles</th>
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</tr>
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The average number of alleles per locus among the varieties was 4.2 and, this value was similarly reported by (5) in a collection of 39 determinate and indeterminate tomato inbred lines collected from China, Japan, South Korea, and USA using 35 SSR polymorphic markers. Variation reported in the number of alleles of tomato varieties and their mean value may be related to variation in the loci studied as well as the number of genotypes and the locations or due to wild range of origin varieties used in these studies (19).

The values of heterozygosity for each locus varied from 0.63 for Tom 31-32 locus to 0.89 for Tom 49-50 locus with a mean of 0.30. Heterozygosity is important to measure the genetic diversity of polymorphic loci. The higher heterozygosity values, the broader the genetic diversity (24). Three out of five SSR primer (Tom8-9, Tom41-42, Tom67-68) produced scorable and reproducible were monomorphic even when using modified amplification conditions.
The highest PIC value was observed in primer Tom 49-50 (0.58) and the lowest PIC values was observed in primer Tom 31-32 (0.45), with an average of 0.21.

The PIC values, a reflection of allele diversity and frequency among the varieties (19). The PIC value (0.58) determined in this study agreed with a study of thirty sex of tomato inbred lines collected from China, Japan, S. Korea, and USA based on SSR marker (5). Other study report higher PIC value reaches up to 0.81, the increase of PIC value even up to 0.7 very useful for variety identification and genetic assessment of tomato germplasm using SSRs markers (19).

Allelic variation might be correlated with the number of repeats within a particular microsatellite locus (SSRs comprised of dinucleotide repeats had the highest PIC value). PIC value in dinucleotide repeats was ranged between 0.45-0.58 while in trinucleotide repeats was (0.0). A relationship was found between the number of repeats and the PIC in this study and earlier reports in tomato (38; 16) and other crops like maize (11) and avocado (3).

The expected reason for these results was that dinucleotide repeats have been reported to reside outside coding regions of genes (40) and are characterized with more repeat numbers (22) making them the best source of highly polymorphic SSR markers. In contrast, trinucleotide repeats are more abundant in protein coding regions (42) with relatively small repeat numbers and total length (41; 27).

Figure 1: The amplification of the SSR primer Tom 31-32, lane M: DNA ladder and lanes 1-19: tomato varieties.
Figure 2: The amplification of the SSR primer Tom 49-50, lane M: DNA ladder and lanes 1-19: tomato varieties.

Genetic distance (or similarity) can reveal the genetic diversity of individuals (36). The genetic relationship between the varieties was determined using the genetic distances. This difference between the two varieties can provide a good estimate of how divergent they are genetically (4). The genetic relationships between the genotypes were estimated with (24) standard genetic distance (GD). Other authors also made use of (GD) estimates (48). The matrix for genetic distance estimates is shown in Table(3).

The lowest genetic distance was (0.3244) between varieties Kenanh (lane5) and Warda (lane10).

Table 3: The genetic distance values between tomato varieties studied in SSR analysis.

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</table>
These results suggested that the tomato varieties in different origins may have some common genetic bases. Thus, it would be possible for gene flow between different geographic distributions.(12).

The highest genetic distance was (0.9177) between varieties Helam (lane3) and Super marimond (lane 16) which means that the presence of similarity between them are very low and they were collected from different geographical origins (Germany and France respectively).

The genetic similarity values ranging from (0.6756 to 0.0823) depending up on the genetic distance values ranging from (0.3244 to 0.9177), which indicate the substantial diversity (32% to 91%) among the varieties used for this study.

(5) reported that the average of genetic similarity among 39 tomato inbred line could reach 0.71 using 35 SSR polymorphic markers.

A collection of ten cultivars of tomato grown in Egypt were screened with 20 simple sequence repeat (SSR) primers in order to determine genetic identities, results revealed Similarities among the ten tomato cultivars ranged from 17.6 to 93.2%(10).

(9) noted that the reason for the differences in the genetic distance between varieties due to the effect of the environments.

Molecular markers are very useful in studying the relationship of closely related lines as they allow calculation of genetic distance based on allele frequencies. The SSR markers are usually scored in terms of presence or absence of a band which can be described as a binary variable. There are several methods to calculate distance/similarity coefficient of paired varieties (23 and Johnson and 17). Dendrogram construction relies on a clustering method and a distance coefficient (25).

(34) recommended Neighbor–Joining method (NJ) to construct dendrograms based upon the closest neighboring pair that minimizes the total branch length of operational taxonomic units (OUTs).
The UPGMA clustering method was used assuming a constant rate of evolution. In contrast, NJ method is based on the assumption of the minimum evolution (26). However, (25) suggested that the accuracy of a phylogenetic tree depends on tree construction method and distance coefficients, and more than one method should be employed for phylogenetic tree construction (20).

Phylogenetic tree was constructed to determine the genetic differentiation. The phylogenetic relationship between nineteen varieties of tomato was determined from genetic distance estimation. The neighbor-joining method was used to obtain the unrooted tree (Figure 4).

Analysis of results obtained from genetic distances (Table3) and Neighbor-joining dendrogram (Figure 6) revealed that, grouped all the 19 tomato varieties into two major groups: first cluster include 9 varieties distributed in two subgroups: the first subgroup include 4 varieties: Mongal (15), GSN(1), Bushra(9) and Helam(3). The second subgroup containe 5 varieties: Super regina(12), Super queen(17), Shady lady(7), Dalal(8) and Kenanh(5). The second major cluster include 10 varieties distributed between two subgroups. The first subgroups include one variety: shahirah(18) while the second subgroup include 9 varieties: Special pack(14), Supermarimond(16), Oula(4), Carioca(13), Sanam(2), Tamara(19), Warda(10), Fotton(11), Douna(6).
In the phylogenetic analysis, most of the tomato varieties were not clustered together in respect to their geographical origin, and thus, might have a similar genetic background. (12) reported that tomato varieties that belong to Lycopersicon chilense clusters in dendrogram by SSR markers in one group.

Similar investigation of genetic diversity among 67 of Argentina tomato varieties using SSRs revealed that there was no relationship between the clustering pattern and the geographic origin of the material (49).

(50) confirmed that in SSRs phylogenetic analysis of twenty of maize varieties, most of the varieties were not clustered together in respect to their geographical origin, and conclude that clustering pattern they share similar genetic background.

(39) observed that their is no correlation between geographical origin of cultivars and classification.

(5) reported in a study of 39 tomato inbred line that in the phylogenetic analysis, most of the tomato inbred lines were clustered together in respect to their geographical location and this mainly concerned with their similar genetic background.

Conclusions

SSR can reliably be used for the estimation of genetic diversity in crops of commercial importance like tomato.

References


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التنوع الوراثي لبعض أصناف الطماطة (Lycopersicon esculentum Mill.) باستخدام واسمه الانتقائي المتكرر البسيط (SSRs)
الخلاصة

أجريت الدراسة الحالية لتقدير التنوع الوراثي ل 19 صنف من أصناف الطماطمة ( المتحدة وغير المحدودة) المستقرة في العراق باستخدام مؤشرات النواة (DNA Markers) ومتغيرات الوراثة البسيطة (PCR) وهو التكرارات المتسلسلة البسيطة (SSRs).

تم عزل دنا المجنين من أوراق الطماطمة التقنية (بعمير أسبوعين) وانتقلت تفاعلات ال PCR فيÍل النواتج الأولى في كل من نقاء و ماتوريات (Tom 8-9, Tom 41-42, Tom 67-68) لكل موقع تراوحت من 0.63 في (Tom 31-32) إلى 0.89 في (Tom 49-50). ووجد أن قيم التباين الزيوتنيتي (heterozygosity) تراوحت بين 0.63 و 0.90 في (Tom 31-32) إلى 0.89 في (Tom 49-50)، وبمعدل قدره 0.30. وقيمة PIC لم تتراوح بين 0.21 و 0.58 في (Tom 8-9, Tom 49-50, Tom 67-68).

توصلت الدراسة من خلال هذا الفحص الجزيئي إلى أن أفضل صنف هو (Tom 8-9 وTom 41-42, Tom 67-68) و(0.58) و (0.0823) بين الصنفين (Tom 8-9 وTom 41-42, Tom 67-68) و (0.06756) و (0.3244) بين الصنفين (ى Tom 8-9, Tom 41-42, Tom 67-68) و (ى Tom 8-9, Tom 41-42, Tom 67-68). وتبين أن نتائج الدراسة تعكس قيم التفاوت الإحصائي بين الصنفين (ى Tom 8-9, Tom 41-42, Tom 67-68) و (ى Tom 8-9, Tom 41-42, Tom 67-68).

ظهر تحليل النتائج التي تم الحصول عليها من الدراسة المجموعة الأولية ضمت 9 أصناف dendrogram ورسمته هذه بدورها إلى مجموعتين فرعيتين: المجموعة الرئيسية (أ gồm 10 أصناف). ونقرس هذه في مجموعتين فرعيتين. أظهر تحليل النتائج أن العلاقات الوراثية بين أصناف الطماطمة لا علاقة لها بالصفي المتهرمية والمنشأ الأصلي.