T.C.
YUZUNCU YIL UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF PLANT BIOTECHNOLOGY

DETERMINATION OF GENETIC RELATIONS AMONG TOMATO GENOTYPES WITH ISSRs MOLECULAR MARKERS IN SULAYMANIYAH REGION

MASTER THESIS

PREPARED BY: Abdulrahman Smail IBRAHIM
SUPERVISOR: Assist. Prof. Dr. Çeknas ERDİNÇ

VAN-2016

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ACCEPTANCE and APPROVAL PAGE

This thesis entitled “Determination of Genetic Relations Among Tomato Genotypes with ISSRs Molecular Markers in Sulaymaniyah Region” presented by Abdulrahman Smail IBRAHIM under supervision of Assist. Prof. Dr. Çeknas ERDİNÇ in the department of Agricultural Biotechnology has been accepted as a M. Sc. thesis according to Legislations of Graduate Higher Education on 25/08/2016 with unanimity members of jury.

Chair:..... Signature:

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This thesis has been approved by the committee of The Institute of Natural and Applied Science on ..…../...../...…... with decision number .....

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THESIS STATEMENT

All information presented in the thesis were obtained according to the ethical behaviors and academic rules frame. And also, all kinds of statement and source of information that does not belong to me in this work prepared in accordance with the rules of theses, were cited to the source of information absolutely.

Signature

Abdulrahman Smail IBRAHIM

ÖZET

SÜLEYMANİYE BÖLGESİNDEKİ DOMATES GENOTİPLERI ARASİNDAKİ GENETİK İLİŞKİLERİN ISSR MARKIRLARI İLE BELİRLENMESİ

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Çalışmanın amacı Süleymaniye bölgesinde yetiştirilen 32 domates (*Solanum lycopersicon* L.) genotipi arasındaki genetik çeşitliliğin ISSR moleküler tekniği kullanarak belirlemektir. Çalışma Yüzüncü Yıl Üniversitesi, Ziraat Fakültesi, Tarımsal Biyoteknoloji Bölümü'nde yürütülmüştür. Yerel domates genotipleri arasındaki ilişkiyi belirlemek için 18 primer kullanılmış ve bu primerlerin genetik ilişkiyi belirlemede etkili olduğu belirlenmiştir. ISSR primerlerinin sonuçları, 15 primerden 65 amplifikasyon oluştuğunu ve bu amplifikasyonların 50'sinin polimorfik olduğunu ortaya çıkarmıştır. Benzerlik matriksi polimorfik bant varlığın bağlı olarak Jaccard benzerlik katsayısı ile hesaplanmış ve UPGMA yöntemine göre kümeleme analizi ile dendogram elde edilmiştir. Dendogramda 3 temel grup oluştuğu belirlenmiştir. Genotipler arasındaki genetik benzerlik (GS) katsayıları 0.261 ile 0.941 arasında değişiklik göstermiştir. En yüksek benzerlik 0.941benzerlik katsayısı ile G7 ve G16 genotipleri arasında olurken, en düşük benzerlik 0.261 ile G15-G24 ve G15-G26 arasında bulunmuştur. Polimorfizmi belirlemek için kullanılan 15 ISSR primeri % 50-100 arasında polimorfizm oranına sahip olmuş ve ortalama polimorfizm oranının % 75.61 olduğu belirlenmiştir. Genel olarak ISSR primerlerinde yüksek oranda polimorfizm belirlenmiş, 3F, Sola5 ve Sola12 primerlerinde amplifikasyon oluşmazken en yüksek oranı % 100 ile ISSR6 ve Sola 11 vermiştir. ISSR primerlerinin PIC değeri ortalaması 0.50 olarak tespit edilmiştir. ISSR yönteminin Süleymaniye'nin farklı bölgelerinden toplanmış domates genotipleri arasındaki genetik çeşitliliği tanımlamada etkili olduğu belirlenmiştir.

Anahtar kelimeler: Domates, Genotip, ISSR, *Solanaceae*, Süleymaniye

ABSTRACT

DETERMINATION OF GENETIC RELATIONS AMONG TOMATO GENOTYPES WITH ISSRs MOLECULAR MARKERS IN SULAYMANIYAH REGION

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The goal of this study was to investigate the genetic variation among thirty two tomato genotypes (*Solanum lycopersicon* L.) grown in Sulaymaniyah using ISSR molecular markers technique. This work was carried out in the Yuzuncu Yil University, Agriculture Faculty, Agricultural Biotechnology Department. Eighteen markers were used to estimate the polymorphism among the local tomato genotypes and were discovered to be a useful tool for genotypes identification. The results of ISSR markers, revealed 65 amplified fragments, 50 of them were polymorphic from using 15 primers. The similarity matrix was computed by using Jaccard similarity coefficients, based on polymorphic bands and dendogram established through UPGMA cluster analysis. The dendogram revealed 3 main groups. Genetic similarity (GS) ranged from 0.261 to 0.941 within studied genotypes. The highest similarity was 0.941 for the genotype pairs G7 and G16. However, the lowest similarity index was 0.261 between G15-G24 and G15-G26. Fifteen ISSR markers used to detect DNA polymorphism gave polymorphism percentage for each primer range between 50–100% with a average polymorphism percentage reaching 75.61%. In general ISSR markers could detect high polymorphism, primer ISSR6 and Sola 11 gave the highest polymorphism percentage were 100%, while primer 3F, Sola 5 and Sola 12 did not give any amplification. The mean of PIC (Polymorphic Information Content) value was 0.50 for ISSR markers. The ISSR technology proved useful in describing genetic diversity among tomato genotypes was collected from different geographical around Sulaymaniyah province.

Keywords: Genotype, ISSR, *Solanaceae*, Sulaymaniyah, Tomatos,

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2016

Abdulrahman Smail IBRAHIM

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SYMBOLS AND ABBREVIATIONS

Some symbols and abbreviations used in this study are presented below, along with descriptions.

Abbreviations	Description
SSR	Simple Sequence Repeat
ISSR	Inter Simple Sequence Repeat
PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic Acid
RAPD	Random Amplification of Polymorphic DNA
EST	Expressed Sequence Tags
AFLP	Amplified Fragment Length Polymorphism
QTL	Quantitative Trait Locus
CAU	Chain Agricultural University
SNP	Single Nucleotide Polymorphism
RILs	Recombinant Inbred Lines
SCH	Second Cycle Hybrid
BGH	Banco de Germoplasm de Hortailças
BAS	Bacterial Artificial Chromosome
G	Genotype
PVP	Polyvinylpyrrolidone
hr	Hour
CTAB	Acetyl Trimethyl Ammonium Bromide
NaCl	Sodium Chloride
HCL	Hydrogen Chloride
NaOH	Sodium Hydroxide
pH	Hydrogen Potential
$MgCl_2$	Magnesium Chloride
$dNTP_s$	Deoxy Nucleoside Triphosphate
UV	Ultraviolet

PIC	Polymorphism Information Content
UPGMA	Unweight Pair Group Method with Arithmetic
rpm	Round Per Minute

Symbols	**Description**
Kb	kilo base pair
ml	Mililiter
ng	Nano gram
µL	Microliter
˚C	centigrade
g	Gram
mg	Miligram
M	Molarity
mM	Milimole
K	Potassium

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1. INTRODUCTION

Tomato cultivation has always been a very important part of agriculture in most countries of the world. Tomato is widely cultivated vegetable crop in the world. It is an important source of vitamin.

Tomato (*Solanum lycopersicum* L.) is one of the economic important crops in Iraq. The tomato genetic resources are important materials for breeding and biotechnology, and determination of their relationships has valuable potential in the tomato industry.

Tomato (*Solanum lycopersicum* L.) is the most important Solanaceae vegetable crop and widespread grown on a large scale commercially for its fresh edible fruit and processed products. Its popularity in worldwide made the crop more commercial and crop industry and hybrids based on their requirements. Breeders developed improved varieties using wild accessions repeatedly which have resulted in narrowed genetic base and difficult to differentiate closely related varieties/hybrids. To address the issues many studies have been conducted to evaluate the genetic variety of tomato using morphological, biochemical and molecular markers (Powell et al., 1996; Tam et al., 2005; Terzopoulosand Bebeli, 2008; Vishwanath et al., 2011). The popularity of tomato and its products continue to rise as it is a good source of vitamin A and C in significant amount.

It is extensively used in salad as well as for culinary purposes and also used for various processed forms include pastes, sauces, pulps, juices, ketchup and as flavoring ingredients in soups, meat or fish dishes (Gosselinand Trudel, 1984). Tomato also used as model plant species to study the physiology and biochemistry of seed development, germination and dormancy (Suhartanto, 2002). The assessment of genetic diversity within and between populations of tomato varieties is measured by using morphological, biochemical and molecular characterization (Antonio et al., 2004). To study genetic diversity among tomato cultivars and related species and landraces (Smolik et al., 2006; Terzopoulosand Bebeli, 2008).

In recent years, an important reduction of genetic variability was observed in several fruit quality traits of the cultivated tomato (*Solanum lycopersicum* L.) (Foolad,

2007). In tomato, some microsatellite markers have been developed (Areshchenkova and Ganal 1999). Simple sequence repeats (SSRs) have shown higher level of polymorphism in cultivated *S. lycopersicum* (Suliman-Pollatschek et al., 2002). Nowadays, several molecular markers are being developed, of which simple sequence repeats (SSRs) or microsatellites are the most widely used types. It is suggested that the variation or polymorphism of SSRs are a result of polymerase slippage during DNA replication or unequal crossing over (Levinsonand Gutman, 1987). Simple sequence repeats (SSR) markers have been successfully adopted to analyze genetic diversity in tomato (Alvarez et al., 2001; He et al., 2003; Frary et al., 2005; Benor et al., 2008). Among the series of DNA molecular markers, the ISSR markers are particularly noteworthy, and are based on the SSR polymorphisms (Morales et al., 2011a). ISSRs or ISA *(Inter-SSR amplification*) molecular markers amplify regions between microsatellite loci (small repeated sequences of 2-5 pairs of bases). This class of markers does not require any prior knowledge about the sequences to be amplified and shows high polymorphism in the material, being very useful in studies of genetic diversity, phylogeny, genomics and evolutionary biology (Reddy et al., 2002). The ISSR is being used by several authors in the molecular characterization of many plant species, such as: tomato (Kamel et al., 2010), rice bean (Muthusamy et al., 2008) and coffee (Masumbukoand Bryngelsson, 2006). The ISSR markers were the most efficient to obtain a large number of polymorphic bands. The repeatability of ISSR-PCR is better than RAPD-PCR because ISSR primers are longer and hence have higher annealing temperatures (Kojima et al., 1998). The inter-simple sequence repeat (ISSR) technique can overcome the above limitations (Reddy et al., 2002; Pharmawati et al., 2004). In tomato, ISSRs have been employed for the assessment of diversity among accessions of various Lycopersicon species and among tomato cultivars (Kochieva et al., 2002; Tikunov et al., 2003). Evaluate the efficiency of ISSR markers in the discrimination of accessions according to their geographical origin collection. Among the all marker techniques, the ISSR are most widely applied and probably because they do not require the knowledge of genome sequences.

Genetic analysis of tomato is essential to enhance the genetic yield potential and maximum utilization of the desirable characters for synthesizing of any ideal genotypes (Kumar et al., 2003). The detection of SSR markers on the genomic map of different

field is beneficial to improve breeding programs of this crop. SSR markers are becoming the preferred molecular marker for variety identification in tomato. Molecular markers can be used not only for estimating the genetic diversity of germplasm collections but also for distinguishing genotypes within population (Kantety et al., 1995).

The application of molecular markers in plant breeding programs facilitates the improvement of many crop species (Williams et al., 1990). The genetic heritage of the tomato was further eroded by the development of vintage and modern cultivars, when much of the original diversity within the cultivated *S. lycopersicum* was lost (Williamsand Clair, 1993). Simple sequence repeats (SSR) or microsatellites are not only very common but also hypervariable among the types of tandem repetitive DNA in the genome of eukaryotes (He et al., 2003). Microsatellite loci can be useful in distinguishing cultivars of tomato, which are genetically very closely related to each other (Smulders et al., 1997). SSR are two powerful DNA fingerprinting techniques. However, these a few reports regarding the evaluation of SSR to study the genetic relationships among the tomato varieties in Chinese market. Molecular markers are generally recognized as a reliable means for the genetic identification among plant genotypes (Kaemmer et al., 1995; Bretó et al., 2003; Claudio et al., 2004; Guptaand Rustgi, 2004; Omrani et al., 2007).

Nowadays, several molecular markers are developed, of which inter simple sequence repeats (ISSRs) are the most widely used types. The nature of the PCR based assay used in their amplification and detection. Simple sequence repeat SSR have been used to analyze the genetic relationships among the cultivated tomato varieties (Powell et al., 1996; He et al., 2003). The tomato, *Solanum lycopersicum* L., which is originated in Latin America, is the second most important vegetable crop and is cultivated throughout the world. The cultivated tomato is a well studied species in terms of genetics, genomics, and breeding (Foolad, 2007).

Genetic diversity in the cultivated tomato is generally low due to the occurrence of population bottlenecks during the domestication and the generation of modern varieties (Rick, 1976). Simple sequence repeats (SSRs) or microsatellites are short (mostly 2–4 bp) tandem repeats of DNA sequences. Other reports demonstrated the

importance of SSR to determine levels of polymorphism and to identify different cultivars (Smulders et al., 1997; He et al., 2003).

Makes the largest contribution to the difference in fruit size between most cultivated tomatoes and their small-fruited wild species counterparts (Alpert et al., 1995). Crosses between tomato cultivars and wild species were used for identification of agriculturally important traits as well as for gene mapping (Eshedand Zamir, 1995). However the number of SSR markers available for molecular breeding is still small and only a limited number of SSR markers have been mapped to the tomato genome (Brounand Tanksley, 1996; Areshchenkova and Ganal 1999). We are currently working a program aimed at the determination of local tomato cultivars from Northern Iraq. We also identified 15 ISSR (Inter Simple Sequence Repeat) markers and these markers have possible applications for MAS in tomato breeding.

In this study, we evaluated of thirty two introduced local landraces and cultivars as well as trial to discovery of some molecular genetic markers associated with Inter Simple Sequence Repeat. We evaluated molecular diversity of determinate collected from thirty two different regions, using (15) ISSR markers. In order to clarify the genetic relationships among several species of tomato plants and their cultivars, ISSRs marker analysis was applied to assess the genetic variety of (*Solanum lycopersicum* L.) in Sulaymaniyah region.

The main objective of this study was therefore to assess the genetic variety and relationship of 32 tomato genotypes locally collected from different regions in from Sulaymaniyah governorate as revealed by ISSR markers.

2. LITERATURE REVIEW

Marker are specific region on gene and Specific fragments of DNA that can be identified within the whole genome. It is known that molecular markers could be useful to verify genetic variability in unknown germplasm and to detect variation between organism or specific region of virus, bacteria and another organisms. Marker could be used to known or detect number of base pairs.

Hai-shan et al. (2006), studied late blight caused by *Phytophthora infestans* the most serious disease of tomato production in China. Studies on the genetics of resistance and identification of molecular markers are very useful for breeding late blight resistant varieties. Identified simple sequence repeat markers and studied the inheritance of late blight resistance associated with resistance allele in tomato (*Solanum Lycopersicon* L.). The results came from an F_2 progeny of 241 plants derived from a cross between 5# inbred line that is susceptible to late blight and a resistant accession CLN2037E. The late blight responses of F_2 plants were tested by artificially inoculation of detached-leaflets in plate and natural infection assayed under greenhouse conditions. Both methods showed that the resistance is dominant and inherited as monogenic trait. Genetic mapping and linkage analysis showed that the late blight resistance gene Ph-ROL was located on chromosome 9 with a genetic distance of 5.7 cM to the SSR marker TOM236.

Fan-juan et al. (2010), examined of genetic difference in 61 tomato varieties was applied by SSR from dissimilar species (*Solanum lycopersicum* L., *hirsutum* Humb L., *pimpinellifolium* Miller L., *chilense* Dun. L., *chmielenskii* L., *peruvianum* Miller L and *parvuflorum* Miller L.). Total 2,062 and 869 pure fragments were magnified by RAPD and SSR, respectively. Alternatively, found more polymorphic products by simple sequence repeat as contrasted to RAPD, i.e., 100 and 43.84%, respectively. Alternatively, a higher worth of the average comparison modulus and lesser polymorphism information content worth were reversed in RAPD (0.79 and 0.407) contrasted to SSR (0.56 and 0.687). Concluded that SSR was a greater operative marker than RAPD to evaluate genetic diversity in tomato successions. Resemblance, the genetic base of tomato diversities in Chinese market was tight.

Shirasawa et al. (2010), developed three types of DNA markers: expressed sequence tag (EST)-derived (SSR) markers (TES markers), genome-derived SSR markers (TGS markers) and EST-derived intronic polymorphism markers (TEI markers) using public genome sequence data in tomato. A total of 2,047 TES, 3,510 TGS and 674 TEI markers were established and used in the polymorphic analysis of a cultivated tomato (*Solanum lycopersicum* L.) 'LA925' and its wild relative *Solanum pennellii* 'LA716', parents of the Tomato-EXPEN 2000 mapping population. The polymorphic ratios between parents revealed by the TES, TGS and TEI markers were 37.3, 22.6 and 80.0%, respectively. Those showing polymorphisms were used to genotype the Tomato-EXPEN 2000 mapping population, and a high-density genetic linkage map composed of 1,433 new and 683 existing marker loci was constructed on 12 chromosomes, covering 1503.1 cM. In the present map, 48% of the mapped TGS loci were located within heterochromatic regions, while 18 and 21% of TES and TEI loci, respectively, were located in heterochromatin. The large number of SSR and SNP markers advanced in this research provide easily handling genomic tools for molecular breeding in tomato.

Korir et al. (2014), the genetic variety and connection of 42 tomato varieties sourced from different geographic regions was examined with EST-SSR markers. The genetic diversity was between 0.18 and 0.77, with a mean of 0.49; the polymorphic information content fluctuated from 0.17 to 0.74, with a mean of 0.45. This indicates a fairly high degree of diversity among these tomato varieties. All the tomato varieties fell into 5 groups, with no obvious geographical distribution characteristics despite their diverse sources. The principal component analysis supported the clustering result; however, relationships among varieties were more complex in the PCA scattered plot than in the UPGMA dendrogram. This information about the genetic relationships between these tomato lines helps distinguish these 42 varieties and will be useful for tomato variety breeding and selection. It is recommended that these varieties be subjected to identification using an SSR-based manual cultivar identification diagram strategy or other easy-to-use and referable methods so as to provide a complete set of information concerning genetic relationships and a readily usable means of identified these varieties.

Rajput et al. (2006), in these study described of 60 RAPD primers were screened and used to prove the reproducibility. Every experiment was executed 50 times by 10

nominees in which the reproducibility of two popular molecular marker techniques was scrutinized. RAPDs substantiated hard to reproduce. Whilst SSR alleles were magnified by all candidates, but small differences in their sizing were got. Expressed as the percentage of RAPD bands gained that were also gotten in replicate data, only 80% reproducibility was got for 60 RAPD primers.

He et al. (2003), progressed and described 129 new microsatellite markers for *S. lycopersicum* L. in response to the limited number of SSR markers presently obtainable. Five hundred DNA sequences of tomato were examined for SSRs and analyzed for the drawing of PCR primers. Of the 158 pairs of SSR primers screened against a set of 19 assorted tomato cultivars, 129 pairs produced the prospective DNA fragments in their PCR products, and 65 of them were polymorphic with the polymorphism information content extent from 0.09 to 0.67. Among the polymorphic loci, 2–6 SSR alleles were exposed for each locus with an average of 2.7 alleles per locus; 49.2% of these loci had two alleles and 33.8% had three alleles. The broad majority (93.8%) of the microsatellite loci contained di- or tri-nucleotide repeats and only 6.2% had tetra- and penta-nucleotide repeats. The set of 19 tomato cultivars were gathered based on the banding patterns generated by the 65 polymorphic SSR loci.

García-Martínez et al. (2006), used 19 (SSR) markers and 7 (AFLP) primer combinations to characterize 48 cultivars of tomato, mainly traditional cultivars. The main types were *Solanum lycopersicum* L. 'Muchamiel', 'De la pera', and 'Moruno'. The robustness of the dendrograms and the discrimination power reached with each marker type was similar. Unique fingerprinting even of the most closely related tomato cultivars could be obtained using a combination of some SSR and AFLP markers. A better grouping of the 'Muchamiel' cultivars was observed with SSR markers, whereas the grouping of cultivars of 'De la pera' type was best achieved with AFLPs.

Todorovska et al. (2014), studied genetic variation in eight Bulgarian tomato varieties and breeding lines (variety Plovdivska Karotina, variety IZK Alya, L21b, L53b, L1140, L1116, L975 and L984) differing in their morphological and biochemical composition was assessed using a highly efficient and low cost fluorescent simple sequence repeat (SSR) genotyping platform. Genotyping was conducted with 165 publicly available microsatellite markers developed from different research groups under a number of projects in tomato (SOL Genomics SSRs, Kazusa TGS and TES,

SLM, TMS and LEMDDNa) among which only five (3.03%) let down to magnify the predictable PCR fragments. Of the remaining markers, 81 (50.62%) were polymorphic in the whole collection of eight genotypes. Among the marker groups used, SLM markers was most polymorphic, followed by TMS and SOL Genomics SSR markers. The average polymorphic information content was 0.196 and the total number of amplified alleles was 299, with a mean of 1.869. The genetic diversity within the collection was relatively low (0.2222). Nei's genetic distance varied from 0.0953 to 0.3992. Cluster analysis using the UPGMA method indicated that the studied tomato genotypes are grouped in four main clusters, which is to some extent consistent with the morpho- and hemo-types of the studied tomatoes. Fourth cluster includes the other five genotypes. The observed grouping of these genotypes in two sub-clusters reflects their similar morphological and biochemical composition.

Mazzucato et al. (2008), studied of phenotypic and genetic diversity in landrace collections is important for germplasm conservation. Here, 50 tomato landraces collected, nine vintage and modern cultivars, and two wild outgroups were grown at two locations in central Italy and characterized for 15 morpho-physiological traits and 29 simple sequence repeat (SSR) loci. The markers were selected to include a group of loci in regions harboring reported quantitative trait loci (QTLs) that aVect fruit size and/or shape (Q-SSRs) and a group of markers that have not been mapped or shown to have a priori known linkage (NQ-SSRs). As revealed by univariate and multivariate analyses of morphological data, the landraces grouped according to vegetative and reproductive traits, with emphasis on fruit size, shape and Wnal destination of the product. Compared to the low molecular polymorphism reported in tomato modern cultivars, their data reveal a high level of molecular diversity in landraces. Such diversity has allowed the inference of the existence of a genetic structure that was factored into the association analysis.

Jia et al. (2009), investigated and evaluated hereditary variation in diverse populations, 216 tomato (*Solanum lycopersicum* L.) cultivars, hybrids, and choice breeding lines from four breeding programs were genotyped using 47 simple sequence repeat markers and disintegrated, 72.3% were polymorphic in all combination of 216 genotypes and 51.06–59.57% indicated polymorphisms in individual populations. CAU population had less unique alleles (2.13%) than further three populations. The cause that

the progress of increasing new varieties with significant improvement of horticultural traits.

Sim et al. (2012), studied influences of picking out on genome variation were examined and envisaged in tomato using a high- concentration single nucleotide polymorphism (SNP) matrix. 7720 SNPs were genotyped on a collection of 426 tomato accessions (410 inbreds and 16 hybrids) and above 97% of the markers were polymorphic in the whole compilation. The array supplied a big number of polymorphic SNP markers across to each sub-population, reaching from 3159 in the vintage successions to 6,234 in the planted cherry successions. Discovered that the level of heterozygosity (proportion of heterozygotes) was depressed, on the other hand changeable between market classes of germplasm. Imagination of underage allele frequency exposed areas of the genome that excellent three representational sub-populations of cultivated tomato.

Xiaorong et al. (2012), studied twenty-six morphological traits in addition to 47 SNP and SSRs were used to examine genetic difference in 67 tomato (*Solanum lycopersicum* L.) varieties collected from Argentina between 1932 and 1974. Nearly 65.0% of the morphological traits and 55.3% of the molecular markers displayed polymorphisms in the 67 varieties. Cluster analysis showed that 67 varieties could be grouped into three clusters at both morphological and molecular levels. Suggested that there was no relationship between the clustering pattern and the geographic origin of the material. The frequency of SNP markers detecting polymorphisms in cultivated tomatoes was higher than any other markers reported to date prop up that SNP markers was useful to describe the genome-wide allelic variation in tomato.

Shats. et al. (2002), studied to generation of SSRs and SNPs and their mapping, together with AFLP loci, to the tomato genome make use of the introgression lines. Primers were draw up plans for 114 loci and utilized for genotyping 13 tomato varieties and three tomato species. Eighteen markers were used to estimate the polymorphism among the commercial cultivars and were discovered to be a useful tool for cultivar identification. The low level of length polymorphism of SSR loci discovered in tomato in this research is in contract with data from other studies. Discovered the fact that the tomato is a self-pollinated crop. Twenty-five SSR markers (48%) were mapped to ILs having centromeres which is a sign for clustering of SSRs in these regions. It is

noteworthy that long GATA, AT, GA and GT reiterates were discovered to be above crowded near centromeres. The average SNP frequency was discovered to be one in a little tens of base pairs. Overall of 52 microsatellites, 159 polymorphic AFLP markers and six SNPs were mapped using the Introgression Lines.

Vishwanath et al. (2013), studied to assess the usefulness of diverse markers system for analyzing the genetic diversity and relative between dissimilar varieties and to discovery out association between marker systems revealed. Totally examined tomato cultivars could be discriminated from each other based on either morphological/protein/RAPD markers individually, and can be practical for combination of cultivars, lineage analysis and genetic diversity analysis. Figure out the some of the studied cultivars were closely related and some of chosen cultivars might have related lineage origin which was due to contentious breeding programmer which necessitated us to relay up on other techniques like protein / isozyme.

Naz et al. (2013), studied to selection programed for breeding. Analyzed all tomato accessions by two parameters e.g. morphological and molecular parameters. For morphological description the plant height, shape, size, leaf length and width, and fruit size, color and shape were selected and for molecular description RAPD markers were used. Overall 25 RAPD defamer primers were selected for the genetic analysis of all tomato accessions. Only 15 polymorphic RAPD primers were accessed for the genetic distance calculation to discover out the phylogenetic association among 25 tomato accessions. The average genetic similarity perceived across all the genotypes was 75.6% with 24.4% polymorphism in 25 tomato accessions. The study wanted to characterize diverse tomato accessions in Pakistan through morphological and molecular markers and then the generation of phylogenetic tree for these diversities on the basis of RAPD fingerprints.

Kamel et al. (2010), studied the genetic evaluation on twenty three genotypes of tomato (*Lycopersicon esculentum* Mill.) under elevated temperature at summer season to be determined the variation between them for heat tolerance. Heat tolerance connected gauges, i.e., pollen viability, fruit setting, osmotic pressure and fruit yield per plant. The pollen grain viability and fruit setting criteria deliberate as appropriate morphological markers for heat tolerance than other heat tolerant associated principles as osmotic pressure. Heritability was elevated and temperately, whereas the genetic

amelioration of new strains could be done. Developed some molecular genetic markers related with heat tolerance in tomato by using ten RAPD and six ISSR primers. Two RAPD markers and one ISSR marker were considered as dependable markers for heat tolerance as well as promptly genotypes possessed eight RAPD markers.

Edris et al. (2014), studied eight mercantile cultivars of tomato (*Solanum lycopersicum* L.) grown in Saudi Arabia and opposing in fruit improvement dates were described on the molecular genetic basis by ISSR and AFLP markers. The study revealed an extensive range of molecular variation, in which some markers characterized between dissimilar genotypes. In common, together sets of data permitted for the identification of cultivars by intends of pairwise variances, cluster analysis and major component analysis. AFLP and ISSR approximates enabled distinction among the eight tomato cultivars.

Mahuad et al. (2013), studied the sequence- connected amplified polymorphism was used as the molecular method for detecting polymorphism among these 18 genotypes. The calculation of polymorphism in recombinant inherited lines (RILs) and second cycle hybrids (SCH) was 61% and 66% separately. Numerous multivariate analyses were performed to discovery agreement between the phenotypic variability observed for fruit superiority traits and the polymorphism gotten from sequence-related amplified polymorphism markers. A general Procrustes analysis assessed that there was a consensus percentage of 75% between phenotypic and molecular data. The experiments discovered that the new hybridizations show new type of variability for fruit quality traits.

Aguilera et al. (2011), studied to evaluate the genetic variability of 96 tomato accessions by used ISSR molecular markers from *Banco de Germoplasma de Hortaliças* (BGH) of *Universidade Federal de Viçosa*, Minas Gerais, Brazil. Ten ISSR primers were individually magnified to let the distinction of the material. All ten primers engendered 144 DNA bands, 53 being polymorphic, with an average of 14.4 per primer. Succession BGH-980 was situated in a dispersed group, being the greatest separate amongst the successions examined by both methods. DNA profiles based on ISSR markers exposed the potential of the digital fingerprints in the analysis of wholly accessions. ISSR markers have a high efficacy to differentiate the germplasm of wild species and useful in the description of *S. lycopersicum* accessions, amplified a

comparatively large number of loci per primer. Some differences among the accessions composed in Brazil and in the USA with the same standard classification in most successions.

Francesco et al. (2014), studied the diversity of a long shelf-life Italian tomato (*Solanum lycopersicum* L.) landraces collection has been assessed using both morphological traits and molecular markers. Besides morphological traits, molecular markers are a powerful tool to discriminate for genetic diversity in crops, especially the microsatellites (SSRs) able to discriminate between homozygous and heterozygous genotypes. The principal component analysis displayed a big cluster in which approximately all landraces from Sicily were comprised. The values of microsatellite polymorphism displayed a low genetic variability amid these long shelf-life tomato cultivars. Cluster analysis based on 10 polymorphic SSR was not able to discriminate landraces for their different origin, while allowed to classify analogous genotypes in four groups.

Nadra et al. (2013), evaluated genetic diversity of 11 tomato (*Solanum lycopersicum* L.) varieties were evaluated through RAPD analysis. Twenty random oligonucleotide primers used in the RAPD-PCR produced a sum of 584 dissimilar marker bands with an average of 29.2 bands per primer. Based on the banding pattern 94.168% polymorphism perceived amid the tomato varieties. Size range of amplified DNA bands varied from 0.1 - 10 kb. A overall of 15 unique bands were amplified from the genome of the 11 tomato varieties. The values of pair-wise genetic distances ranged from 0.1838 - 0.9049, indicating the attendance of extensive genetic diversity.

Aida et al. (2015), studied genetic relationship between three landraces of tomato (*Solanum lycopersicum* L.) from Sudan in relation to a heat sensitive commercial genotype. Detected DNA polymorphism by using RAPD markers were used 16 primers. This experiment showed that the 16 primers gave polymorphism percentage for each single primer range between 0 – 83% with a total polymorphism percentage reaching 39%. Primers OPB-0 2, OPA- 10 and OPB- 20 gave the highest polymorphism percentage in a range of 71 - 83% while OPC-07 did not give any polymorphic fragments.

Parmar et al. (2013), found of molecular markers and marker assisted selection technology, the research has inserted into a recent era and had make it possible to grow new and most informative PCR-based markers, including SSR, and to ulterior expedite the use of markers in tomato breeding. This experiment is a pace to introduce a new SSR marker (TOM-144) which was inferred after appraisal of eight microsatellite loci amongst the twenty-one different tomato cultivars. The marker selected was inherited and isolated in men delay in fashion as demonstrated consecutive generation of a cross among parent cvs. H-24 x GT-2.

Lana et al. (2012), progressed a set of practical SSR markers via *in silico* analysis of overtly available tomato DNA sequences. Development of microsatellite to map-referenced is a very effective. Progressed 17 SSR markers and tested on one tomato mercantile cultivar and eight local landraces. Twelve loci (27 alleles) were recorded and displayed 100% polymorphic patterns. The calculated polymorphism information content worths for the SSR markers progressed ranged from 0.62 to 0.97. The SSR motifs CT (26) AT (27) and TTC (6) TTA (4) had the highest PIC worth (0.97), while CAA (5) A (8) had the lowest PIC worth (0.62).

Mohamed et al. (2012), studied 20 microsatellite primers screened with collected of ten cultivars of tomato developed in Egypt. Intermediary, 38 alleles were improved using SSR primers with fragment sizes ranging as of roughly 75 to 275 bp. 23 alleles were polymorphic thus divulging 60.5% of polymorphism. The genetic similarity assessed according to SSR data was gauge up among 17.6 and 93.2%. Definite identification of cultivars of tomato by SSR. It mean cultivars Manapal had Roma –VF (one negative) and (two positive and four negative). UPGMA clustering grouped the cultivars into two groups where Giza 80 were clustered in diverse group and the two Egyptian cultivars Edkawy.

Maria et al. (2013), studied to amount genetic variety present in 47 greatest joint tomato varieties (*Solanum lycopersicum* L.) grown in Italy. Variety was gauged using 94 genotypes and 11 microsatellite markers. Showed the potentiality that the variety traceability to be able stretched through the whole production chain. The researchers of 48 alleles among using the markers. A dendrogram based on whole simple sequence repeat polymorphism assembled 47 varieties into three main clusters at 0.75 likeness coefficient, distinguishing the recent varieties from tomatoes landraces. The DNA

markers progressed confirmed the potentiality to back the genotype identification all the length of the tomato production chain. Recognized genotypes and the number of alleles in the present work is the biggest mull over papers on food traceability.

Pritesh et al. (2010), collected of twenty-five determinate and indeterminate cultivars of tomato from different geographical locations of India and screened them with twenty-three SSR (simple sequence repeat) primers in instruction to decide genetic identities, genetic associations and genetic variety between these cultivars. 40 alleles were magnified using SSR primers with storable fragment sizes ranging from roughly 150 to 1000 bp. UPGMA clustering assembled the cultivars into five groups with the USA cultivars forming a distinct group.

Zhou et al. (2015), developed and applied the utilization of 48 tomato cultivars by using the gSSR and EST-SSR markers for analyzing genetic variability among tomato cultivars from America, China, the Netherlands, and Portugal. In all, 15 of 82 gSSR and 18 of 115 EST-SSR markers displayed polymorphic loci. There were 995 and 2072 clear fragments amplified by polymorphic gSSR and EST-SSR markers, respectively. All of alleles revealed by EST-SSRs (75, 4.2) was above the gSSRs (54, 3.6). A lower polymorphism information content value was found in gSSRs (0.529) compared to EST-SSRs (0.620). Likeness coefficient matrixes of the 48 tomato cultivars was a high similarity were perceived between the gSSRs and EST-SSRs dendrograms.

Saida et al. (2013), studied 19 Azerbaijan tomato genotypes analyzed by RAPD. A total of 26 amplified products were revealed by 6 primers. The genetic similarity among evaluated genotypes ranged from 0.188 to 1.000. The lowest similarity was observed between cultivars ‘Azerbaijan’ and ‘Shakar’ (0.188), while the highest between ‘El-nur’ and ‘Garatag’ (1.000). UPGMA cluster analysis based on Jaccard’s similarity coefficient divided genotypes into four main groups. The first group was the largest and consisted of 12 genotypes, while the fourth group was the smallest consisted of 1 genotype only. Calculated average genetic diversity from RAPD data was 0.665.

Ning et al. (2012), studied and developed on the foliage mould defiance gene *Cf*-10 on tomato were used SSR and AFLP molecular markers. Discuss resistant or immune to all predominant physiological races of *Cladosporium fulvum* presented in three northeastern provinces of China in pollination experiment. Evaluated the

preferable utilize *Cf*-10 gene in a marker-assisted selection program and to license the pyramiding of one or several resistance genes in a cultivar, compactly connected SSR and AFLP markers were got by the bulked segregate analysis method. One SSR marker and three AFLP markers were recognized connected to *Cf*-10 gene, with the range of 9.73, 5.8, 8.5, and 10.6 cM, respectively. These markers would ease the selection of resistant tomato germplasm containing *Cf*-10 gene.

Brounand Tanksley (1996), studied simple sequence repeats (SSRs) used to screen of tomato genomic libraries with seventeen synthetic oligonucleotide probes, consisting of 2- to 5-basepair motifs repeated in tandem. GAn and GTn sequences were found to occur most frequently in the tomato genome (every 1.2 Mb), followed by ATTn and GCCn (every 1.4 Mb and 1.5 Mb, respectively). Figure out polymorphism of microsatellites was measured by PCR amplification and used a set of ten tomato cultivars. Surprisingly, only two of the nine microsatellite clones surveyed. Seventeen oligonucleotide probes identified GATAn and GAAAn by using Southern analysis among tomato cultivars and determined structure of microsatellite loci. Southern hybridization was detect nine large hypervariable fragments with a GATA8 probe.

Areshchenkova and Ganal (1999), isolated tomato microsatellites containing long arrays (> 20 repeats) of the dinucleotide motifs GA, GT, AT, as well as GATA, assessed their variability within *Solanum lycopersicum* L. varieties and mapped them onto a genetic map of tomato. The investigated microsatellite markers exhibited between 1 and 5 alleles in a diverse set of *Solanum lycopersicum* L. lines. Mapping of the microsatellites onto the genetic map of tomato demonstrates that, as previously shown, GATA microsatellites are highly clustered in the regions of the tomato centromeres. Described some microsatellite markers could be more suitable tool to molecular structure of tomato centromeric regions, for variety identification and for mapping experiments.

Geethanjali et al. (2010), evaluated SSR markers relating to chromosome 6 from bacterial artificial chromosome (BAC) sequences obtainable at Solanaceae Genomics Network. A complete of 54 SSR primer pairs from 17 bacterial artificial chromosome clones on chromosome 6 were draw up and validated. Polymorphism of these loci was estimated in a panel of 16 genotypes including of *Solanum lycopersicum* L. and its wild relations. Twenty-one SSR markers obtained from 13 BAC clones were polymorphic

between two closely associated tomato successions, West Virginia 700 and Hawaii 7996 were drew using a recombinant inbred line population to get from a cross between these two accessions. The markers were divided throughout the chromosome spanning an overall length of 117.6 cM following the instruction of the original BAC clones.

Bredemeijer et al. (1998), studied to assess the benefit of a fluorescent-analysis method for genotyping PCR-based tomato microsatellite markers (or STMSs) and to set up the worth of these markers to generate singular DNA profiles of tomato cultivars. Analyzed of the tomato STMSs exposed distinct allelic peaks. Contrast of fluorescent and silver-stained allelic profiles exposed an analogous distribution of alleles among the test cultivars. Sixteen tomato cultivars were DNA-typed for 20 picked STMS markers using the fluorescent approach. Length polymorphism among the PCR products was uncovered with 18 of these markers, yielding gene diversity worth from 0.06 to 0.74. The number of alleles per microsatellite locus ranged from 2 to 8.

Alvarez et al. (2001), studied to determine informative a set of microsatellites from tomato is across the genus (*Solanum Lycopersicon* L.)17 microsatellite loci and tested on 31 accessions comprising the nine species of the genus. Estimated distribution of diversity throughout the genus and evaluated efficacy of microsatellites for establishing species relationships in comparison with existing phylogeny reconstructions by using microsatellite polymorphisms. Found a high level of polymorphism, as well as a large number of alleles unique for species. Detected microsatellite loci with the level of polymorphism within and among species was highly correlated with the respective mating systems, cross-pollinating species having a significantly higher gene diversity compared to self-pollinating species.

Kwon et al. (2006), examined of SSRs for identification and genetic diversity in 28 commerical tomato varieties. Analyzed genetic resource of tomato by simple sequence markers. Marker genotypes have relationship with 28 varieties was analyzed. The 219 couples of SSR primers screened anti ten tomato varieties, 18 pairs was highly polymorphism with polymorphic information gist extent from 0.467 to 0.800. Detected two to nine SSR allels for each locus with an middling of 3.3 allels per locus among the polymorphic loci.

Tikunov et al. (2003), studied of five tomato species. Fourteen ISSR primers were used to study the five tomato species. Nine of the fourteen primers generated

distinct band fingerprints for every tomato species assayed. Most of the bands were polymorphic. Obtained unique ISSR fingerprints for the accessions of each tomato species.

Kochieva et al. (2002), analyzed ISSR molecular marker used to study the genetic diversity, phylogenetic relationships in 54 wild and applicable for genome marking in the genus (*Solanum lycopersicum* L.) used 14 anchored primers, which were homologous to microsatellite repeats and contained one to three anchor nucleotides. In total, 318 ISSR fragments of the tomato genome were obtained. Sample polymorphism revealed with the ISSR primers was high, reaching 95.6%, were detected for each tomato species. Of these fragments, only 14 fragments were monomorphic, occurring in patterns of all representatives of the genus, and 304 were polymorphic.

Terzopoulosand Bebeli (2008), used 27 inter-simple sequence repeats (ISSRs), 12 yielding polymorphic amplification products were used to the genetic diversity of 33 Greek tomato (*Solanum lycopersicum* L.) landraces and three cultivars, three cherry tomato (*S. lycopersicum* var. *cerasiforme*) cultivars and two accessions of *Solanum pimpinellifolium* L. The ISSR data distinguished all the 27 morphotypes from each other and grouped the morphotypes derived from the same landrace together. The ISSR technology proved useful in describing genetic diversity among Greek tomato landraces and was capable of distinguishing the closely related morphotypes.

3. MATERIAL AND METHOD

3.1. Material

Thirty two tomato landraces (*Solanum lycopersicum* L.) accessions collected from different regions province of Sulaymaniyah-Iraq was used in this study (Table 3.1 and Figure 3.1). Hence, thirty two tomato genotypes seeds grown in open field were received in Sulaymaniyah district around, 36° 15' North latitude and 44° 71' East longitude, 36° 70’ North latitude and 45° 95' East longitude and ect. The elevation of Sulaymaniyah center is about 830 m above sea level, Iraq. Sulaymaniyah is located in northern Iraq. It is characterized by its cooler summer temperatures and its rainy winters. Average temperatures range from 0 ℃ to 39 ℃. In the winters, there can be a significant amount of snow. The city is actually known as the “windy city" in the region, due to the mountains that surround it, in order the type of climate is continental climate Zakaria et al. (2013)

Table 3.1. Genotypes name of tomato used in this study and origin of accession used for ISSR studies

Accession Number	Origin	Accession Number	Origin
G1	Sindolan	G17	Mergapan
G2	Isawi (small size)	G18	Suse
G3	Isawi (big size)	G19	Badawa
G4	Beshir	G20	Beklo
G5	Kfradol	G21	Khdran
G6	Hero	G22	Halsho
G7	Said Ahmadan	G23	Mamanda
G8	Yaran Bagi	G24	Penjuin
G9	Chwas	G25	Dawdya
G10	Sangasar	G26	Bokriskan
G11	Sangaw	G27	Benasa
G12	Shekhawdalan	G28	Salara
G13	Ashkana	G29	Kalar
G14	Shene	G30	Begalas
G15	Bawze	G31	Gira
G16	Saruchawa	G32	Swru

Figure.3.1. Suleymaniyah Governorates.

3.1.1. Plant samples

The sample of the tomato varieties were collected around of Sulaymaniyah province in northern Iraq. All landraces used in this study are genetically pure (Figure 3.2).

Figure.3.2. Fruits of tomato genotypes used this study

Figure.3.2. Fruits of tomato genotypes used this study (continued)

3.1.2. Genomic DNA extraction

Seedlıngs were grown in a growth chamber at a temperature of 24°C with a 16/8 h day/night photoperiod with 60% of moisture. Each replicate included 10 seeds per variety. They were fertilized was at the first of April 2016, using liquid fertilizer (Hoagland). Genomic DNA was extracted in bulk from young fresh leaves (Figure 3.3).

Figure.3.4. Growing tomato seedlings.

3.1.3. DNA isolation

Genomic DNA of each variety were extracted from young leaf tissues sampled at the two-to-three leaf stages employing the CTAB procedure (Doyleand Doyle, 1987). DNA was quantified by Nano Drop, ND 100 spectrophotometer (Nano Drop Technologies, Inc.). DNA were diluted in water to a final concentration of 50 ng/µL and stored at -20°C (Figure .3.4).

Extracted DNA by these steps:

1. Prepare CTAB buffer (see appendix), use within 2-3 days, store capped: Add polyvinylpyrrolidone mol. weight 40,000 (PVP-40) and β-mercaptoethanol and stir to dissolve before starting extractions:

CTAB	PVP-40	β-merc
'] 0.5 ml	0.02g	2.5µl
5 ml	0.2g	25µl
20 ml	0.8g	100µl

2. Weigh out 40-50 mg of frozen leaf tissue and lyophilize -80°C for two days.

4. 500µl of CTAB buffer and grind samples has been added with pestle.

5. Transfer solution to a 1.5 ml tube.

6. 10µl of β-Mercaptoehanol was added.

7. 10µl of Protienase potassium (K) has been added and mix by inverting.

8. Incubate samples at 65°C for 1hr.

9. 500 of 24:1 Chloroform-Isoamyl Alcohol has been added and mix well to form an emulsion by shaking tubes with hands.

10. Centrifuge for 30 minutes at maximum speed (13500 rpm).

11. Pipette off the aqueous phase (top) taking care not to suck up any of the middle or Chloroform phases.

12. Place the aqueous phase into a new labeled 1.5 ml tube.

13. 0.08 volumes of cold 7.5 M Ammonium Acetate was add (see appendix).

14. 0.54 volumes was add (using the combined volume of aqueous phase and added AmAc) of cold isopropanol (=2-propanol).

This should be approximately 204 µl.

15. Mix well.

16. Let sit in freezer for 45 min to an hour.

17. Centrifuge for 5 min at maximum speed (13500 rpm).

18. Pipette off the liquid, being careful not to lose the pellet with your DNA.

19. 700 µl of cold 70% Ethanol has been added and invert once to mix.

20. Centrifuge for 3 min at maximum speed (135,00 rpm).

21. Pipette off the liquid, being careful not to lose the pellet with your DNA.
22. 700 µl of cold 95% Ethanol was add and invert once to mix.
23. Centrifuge for 3 min at maximum speed (135,00 rpm).
24. Pipette off the liquid, being careful not to lose the pellet with your DNA.

    If you can't remove all the ethanol that is ok. It is better to leave some ethanol than risk sucking up your DNA.

25. Dry the pellet in a vacuum centrifuge or on a hot plate at 55°C.
26. Resuspend samples with 100 µl of water (ddH_2O). Allow to resuspend for 1hr at 55°C before using.
27. 4 µl of RNAase has been added and incubate at 37°C for 15 mins, shake gently every 5 mins.

Preparing solutions used DNA isolation.

CTAB: for 1L of CTAB buffer

100 ml of 1 M Tris (pH 8.0)

280 ml of 5 M NaCl

40 ml of 0.5 M EDTA

20 g of CTAB (Cetyl Trimethyl Ammonium Bromide)

To 1L with H_20

1 M Tris, pH 8.0: for 1 L

121.1 g Tris

700 ml ddH_2O

0.5 M EDTA pH 8.0: for 1 L

186.12 g of EDTA

750 ml ddH_2O

5 M NaCl: for 1 L

292.2 g of NaCl

7.5 M Ammonium acetate: for 250 ml

144.5 g ammonium acetate

The quantity and quality of DNA was determine by Nano Drop spectrophotometer to ensure the good quality of DNA

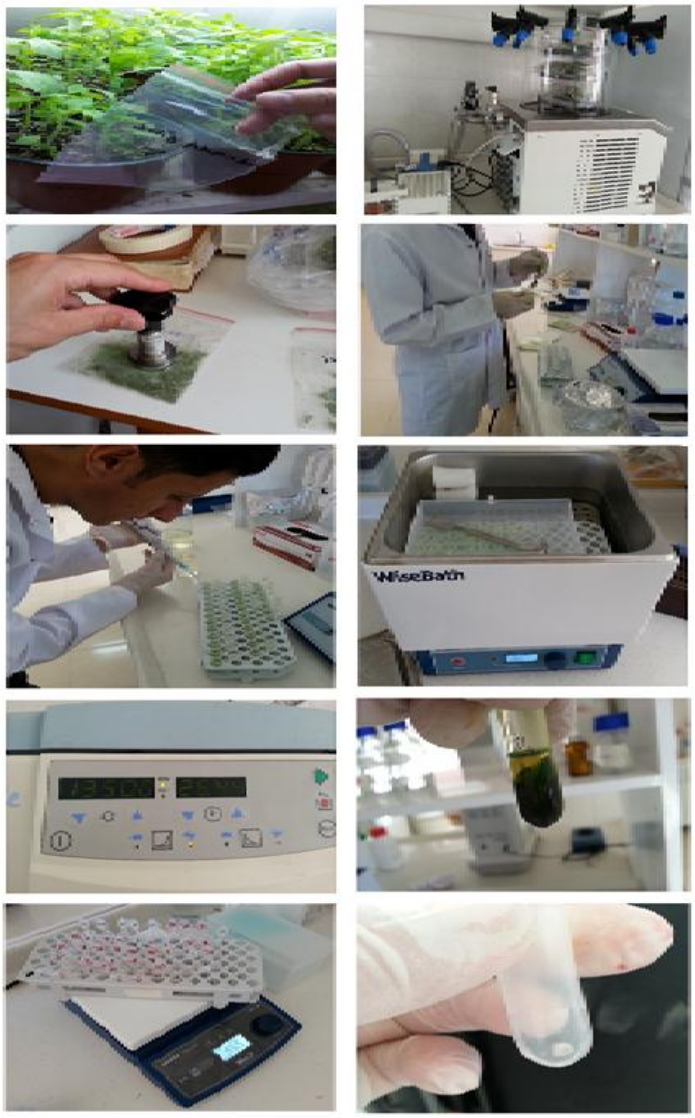

Figure.3.5. Stage of DNA extraction from tomato leaves.

3.1.4. PCR amplification

3.1.4.1. PCR procedure (PCR reactions)

The PCR amplification was performed using a 25µl mixture contained the following components: 8.7µl of sterile ddH2O , 1X Taq buffer, 2.5 mM of $MgCl_2$ (25mM), 0.6 mM of primer , 0.1 mM of dNTPs , 1 unit of Taq polymerase and 20 ng of template DNA.

3.1.4.2. PCR primers

3.1.4.3. ISSR amplification

Fifteen primers of ISSR were utilized in our study is shown in (Table 3.2). For ISSR used four degenerated primers (Tikunov et al., 2003; Terzopoulosand Bebeli, 2008; Aguilera et al., 2011; Figueiredo et al., 2015).

Table 3.2. Sequence data of the ISSR primers applied with number of base and annealing temperature

ISSR primer	Sequence (5'-3')	Annealing temperature(ºC)
12F	$(AG)_8YG$	55.0
13F	$(AC)_8YT$	51.0
16	$(AC)_8$	57.0
812	$(GA)_8A$	51.0
816	$(AC)_8$	52.0
889	$AGTCGTAGT(AC)_8$	57.0
B2	$(AG)_8T$	55.0
ISSR6	$VDV(GT)_8$	55.0
PHV6	$CCA(CT)_8$	57.0
Sola 9	$(AC)_8G$	52.0
Sola11(1)	$GAG-(CAA)_5$	51.0
10F-P4	$(GT)_8YC$	55.0
13	$(CCA)_5$	55.0
825	$(AC)_8T$	52.0
Sola 4	$VHV-(GT)_7G$	55.0
3F	$(GA)_8YT$	55.0
Sola 5	$DBD(AC)_7$	55.0
Sola 12	$CTG-(AG)_8$	55.0

3.1.4.4. PCR amplification program

The ISSR-PCR amplification programs included a denaturing stage at 94 ˚C for 3 minutes, followed by 35 cycles of amplification. Each of the cycle had a denaturing step at 94 ˚C for 20 s, an annealing step at a temperature according the melting temperature of each primer (Table 3.2, Figure 3.5) for 40 s and an extension step at 72 ˚C for 1 minutes. After the last cycle the samples were kept for 10 min at 72 ˚C (Terzopoulosand Bebeli, 2008).

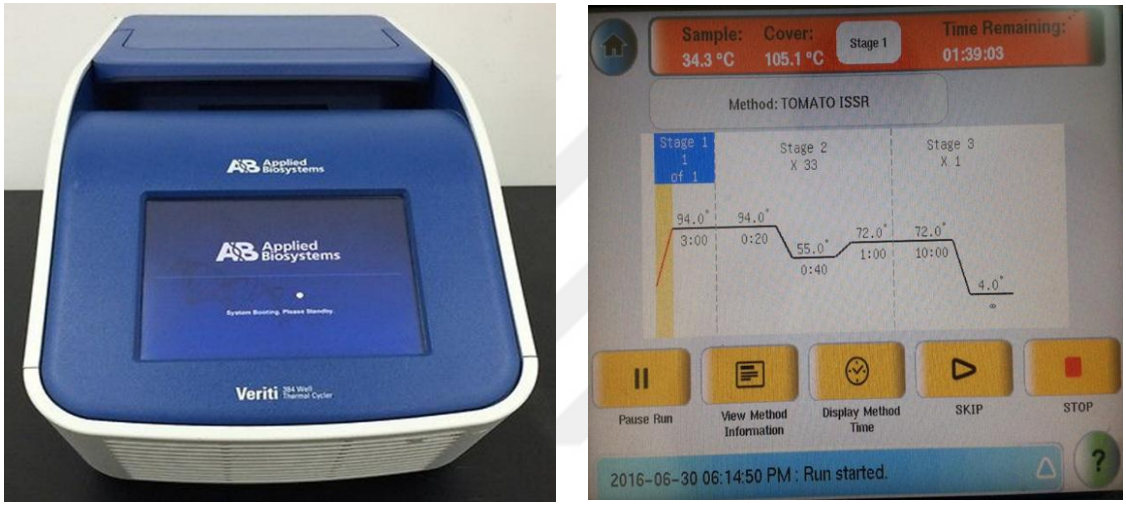

Figure.3.6. PCR cycles and temperature conditions.

3.1.5. Gel electrophoresis

Agarose Gel electrophoresis was performed to separate amplification products: PCR products were loaded on 1.5% agarose gel .The was prepared by dissolving 7.5 g agarose/500 ml of 1X TAE and agarose was dissolved completely by heating in a microwave oven. 25µl of ethidium bromide was used as the staining agent and added to the gel after cooling 50 – 60 ˚C. The above solution was thoroughly mixed and cooled before pouring into gel casting templates fixed with the comb and allowed to solidify. After the solidification of the agarose, the comb was removed and the gel was placed in the electrophoresis unit. PCR products were mixed with the 3µl of agarose gel loading dye and loaded into the gel wells. The gel was run at 160 Volt for 2.5 hr. Gels were

photographed using a Polaroid camera and bands were visualized in the gel under UV light and photographed.

3.1.6. Data analysis

Jaccard similarity coefficients were calculated from the matrix obtained from molecular data. The computer program NTSYpc version 2.11k (Rohlf, 1997) was be used to get dendrogram and cluster analysis. Profile data then summarized by the number of primers used, the number of all fragments, the mean number of fragments per primer/primer pairs, the number of polymorphic fragments, the percentage of polymorphism fragments, the percentage of polymorphic primers, and the average number of alleles. The PIC (polymorphic information content) will be calculated using the following standard formula (Powell et al., 1996; Smith et al., 1997). For phylogenetic analysis, only the data for the polymorphic ISSR loci were entered for all DNA samples, and a “1” or “0” were used if an allele is present or absent for a genotype, respectively.

4. RESULTS

4.1. DNA quality and quantity

Genomic DNA was extract from thirty two tomato genotypes. The quantity and quality of DNA was tested by Nano Drop spectrophotometer to ensure the good quality of DNA for ISSR analysis (Figure 4.1, Table 4.1). To evaluate the quality of genomic DNA sample. The purity of DNA in each sample was determined by usıng Nano Drop spectrophotometer, 260/280 ratio. The quantity of DNA in dissimilar Samples varied from with the ratio of 601 ng to 2019ng. After quantification all the DNA genotypes were chosen for PCR amplification and ISSR analysis. The purity of DNA was determined by 260/280 ratio. Samples with the ratio of 1.57-2.02 were chosen for PCR amplification.

Figure 4.1. Determination of the DNA quantity and quality with Nanodrop.

Table 4.1. DNA quantity and quality of tomato gentypes

Accession number	Quantity of DNA (ng)	Quality of DNA (A 260/280)
1	944.1	1.91
2	1077.1	1.67
3	994.7	2.02
4	920.1	1.89
5	787.7	1.73
6	1075.5	1.82
7	1008.3	1.88
8	1094.4	1.95
9	483.4	1.57
10	994.6	1.84
11	864.3	1.78
12	1438.8	1.80
13	704.2	1.76
14	1133.3	1.84
15	1550.4	1.87
16	605.9	2.00
17	601.9	1.60
18	1152.0	1.90
19	1125.4	1.77
20	1838.5	1.84
21	1031.0	1.74
22	1940.6	1.77
23	1561.4	1.90
24	1568.0	1.91
25	1631.4	1.58
26	680.9	2.00
27	1686.7	1.88
28	2019.0	1.95
29	1160.2	1.86
30	989.2	1.86
31	1210.4	1.71
32	968.9	1.73

4.2. Polymorphism by ISSR analysis

In general in this study used eighteen ISSR primers, among them only fifteen of them were verified and acceptable for experimenting on. A total 65 number of DNA fragments per ISSR primers was detected and were scored from the photographs (Table 4.2). The total amplified fragments were 65 bands, 50 of them were polymorphic bands and 15 monomorphic bands. A high number of DNA fragment in ISSR were 8 per primer 816, while a lowest number of DNA fragments were 2 per primers (sola 11 and 10F). On the other hand, 2 ISSR primers showed high polymorphic products. Figure 4.2 shows parts of profile by ISSR primer 13F, simultaneously the lowest number of

polymorphic band in ISSR were 1 per primer 10F. The average polymorphic band number 3.33 were obtained (Table 4.2). Polymorphism percentage for each single primer was calculated for the fifteen ISSR primers. The polymorphism percentage was range between 50 – 100% for each single primer. The average polymorphism reaching 75.61%. Primer 10F gave the lowest polymorphism percentage were 50% while the highest polymorphism percentage in ISSR6 and Sola 11 were 100%.

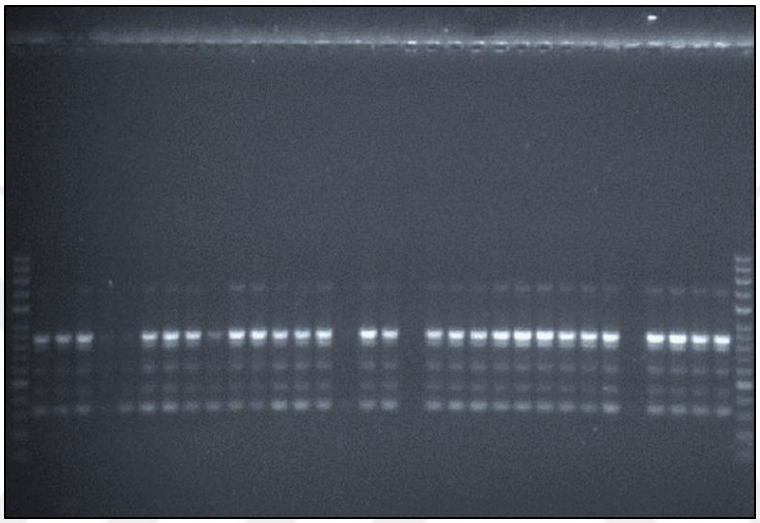

Figure 4.2. ISSR profiles of genomic DNA of the thirty two genotypes of tomato (*Solanum lycopersicum* L.) amplified with primer 13F.

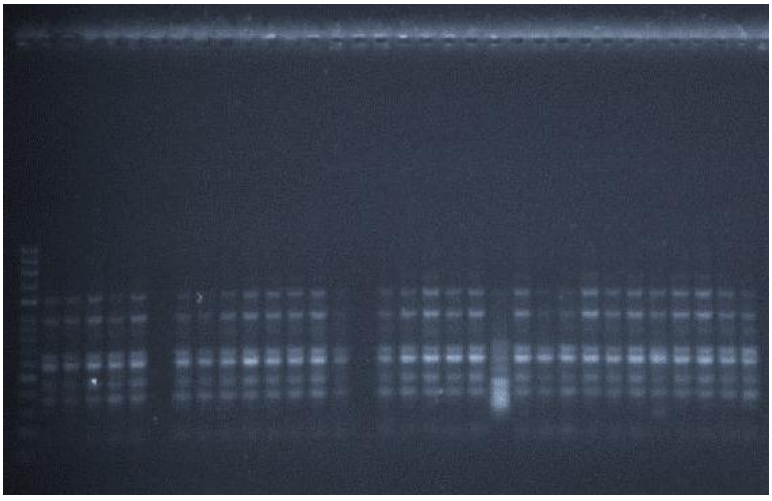

Figure 4.3. ISSR profiles of genomic DNA of the thirty two genotypes of tomato (Solanum lycopersicum L.) amplified with primer 16.

Table 4.2. Number of band, polymorphism and PIC values of ISSR primers used this study

No.	ISSR primer	Sequence (5'-3')	Total number of DNA fragments	Number of polymorphic DNA fragments	Polymorphism (%)	PIC value
1	12F	$(AG)_8YG$	6	4	66.67	0.64
2	13F	$(AC)_8YT$	6	5	83.33	0.21
3	16	$(AC)_8$	6	5	83.33	0.20
4	812	$(GA)_8A$	3	2	66.67	0.86
5	816	$(AC)_8$	8	7	87.50	0.61
6	889	$AGTCGTAGT(AC)_8$	6	4	66.67	0.64
7	B2	$(AG)_8T$	3	2	66.67	0.89
8	ISSR6	$VDV(GT)_8$	4	4	100.00	0.34
9	PHV6	$CCA(CT)_8$	3	2	66.67	0.06
10	Sola 9	$(AC)_8G$	4	3	75.00	0.33
11	Sola11	$GAG-(CAA)_5$	2	2	100.00	0.25
12	10F-P4	$(GT)_8YC$	2	1	50.00	0.95
13	13	$(CCA)_5$	5	4	80.00	0.20
14	825	$(AC)_8T$	3	2	66.67	0.82
15	Sola 4	$VHV-(GT)_7G$	4	3	75.00	0.48
	Total		65	50		
	Mean		4.33	3.33	75.61	0.50

PIC= Polymorphic Information Content.

4.3. Cluster analysis

The similarity index obtained for each pair wise comparison among the 32 tomato accessions using ISSR primers. Jaccard similarity coefficient based on fifteen ISSR markers between all the accessions ranged from 0.261 to 0.941.

The most different tomato genotypes were G15 (0.390), G28 (0.553) and G9 (0.641), while the most similar tomato genotypes were G11 and G17 (0.775 and G20 (0.774). The lowest genetic distance was observed among pair of accessions G15-G24 and G15-G26 (0.261) and G15-G30 (0.269), while the highest genetic distance was noted in G7-G16 (0.941), G27-G29 (0.933) and G20-G27 (0.930) based on combined

ISSR analysis. The three genotypes G15, G28 and G9 are had much higher distances. (Table 4.3, Figure 4.4).

Cluster based on dendogram

Cluster analysis was performed on the similarity index calculated using 15 ISSR markers by UPGMA based on Jaccard coefficient. According to genetic distance matrix denrogram divided to three main groups: Group one it is G15, group two G28, group three divided to two sub groups and each sub groups of them contained sub-mini groups will be separated.

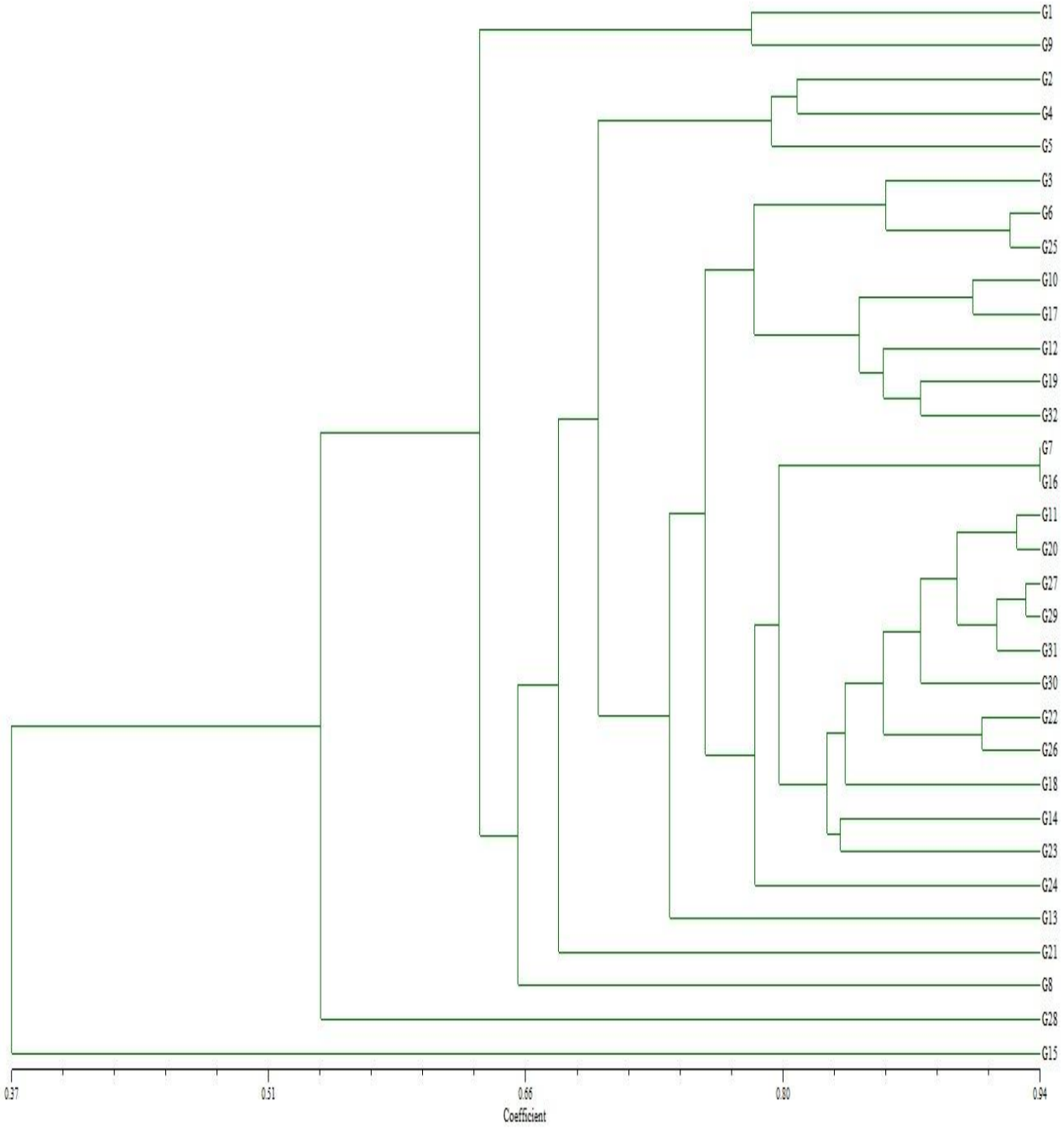

Figure 4 4. Associations among around Sulaimaniyah Basin tomato genotypes revealed by UPGMA clustering analysis on the basis of the molecular Jaccard distance values.

5. DISCUSSION AND CONCLUSION

In the present study, we used eighteen ISSR molecular markers, only fifteen ISSRs were used to evaluate the thirty two tomato genotype in tomato (*Solanum lycopersicun* L.) We obtained 50 polymorphic bands from those primers. In this experiment most of the bands were polymorphic. ISSR primers gave the fingerprints a polymorphism level of more than 50% from 66.67 to 100% of polymorphic bands, while Tikunov et al., (2003) found a polymorphism level of more than 50% from 60 to 89% of polymorphic bands. Fifteen markers were used to estimate the polymorphism obtained (75.61%), while Shats. et al., (2002) reproduced the polymorphism as 65%. We detected high value of polymorphism in tomato by using the application polymerase chain reaction (PCR) based markers such as inter simple sequence repeat (ISSR) revealed 100%, while Kochieva et al., (2002) found the high value of polymorphism as 95.6%.

DNA fragments bands were obtained from each ISSR single primer except the primers (3F, Sola 5 and Sola 4). All of the primers used to this experiment could not produce any DNA fragments, while they are used with the application polymerase chain reaction. When we compared the primers in this study, we realized the primer 816 is the most productive primer with the highest DNA fragment bands, while the lowest DNA fragments we obtained from Sola 11 and 10F primers.

According this study, some of primers (12F, 13F and 16) produced 6 DNA fragment bands. Some primers (ISSR6, Sola 9 and Sola 4) produced 4 DNA bands. The primers (812, B2, PHV6 and 825) produced 3 DNA bands, and the primers Sola 11 and 10F produced 2 DNA fragment bands, except the primers (13 and 816) produced 5 and 8 DNA fragment bands, respectively. The average number of DNA fragments was 4.33.

The number of polymorphic bands by ISSR-PCR application ranged from 1 to 7 with an average of 3.33. Alternatively, Fan-juan et al., (2010) found more polymorphic products ranged from 4 to 18 with an average of 7.91. Among the marker groups used, ISSR markers was most polymorphic with a total 50, while the total polymorphic was 81 in another experiment (Todorovska et al., 2014). Envisioned a different polymorphic bands that obtained from each single ISSR primers, while some of these primers could produce same number of polymorphic bands. The highest number of polymorphic band

was 7, obtained from primer 816, while the lowest number of polymorphic band from 10F primer was 1. Four polymorphic bands were obtained from primers (12F, 889, ISSR6 and 13). Five polymorphic bands were obtained from each primers 13F and 16. Three polymorphic bands were obtained from primers Sola 9 and Sola 4 and two polymorphic bands were obtained from primers (812, B2, PHV6, Sola 11 and 825).

Most of ISSR primers exposed high degree polymorphism was 100% and was successful to amplified the genotypes of tomato visibly, while high degree polymorphism was also in another study 100% (Kochieva et al., 2002). Some of ISSR primers were used to this research gave same percentage of polymorphism. (Korir et al., 2014). It has been confirmed that the EST-SSR marker system is useful for studying genetic diversity among tomato genotypes or cultivars. The high degree of polymorphism and the large number of bands obtained per assay showed that ISSR is the most informative marker system for tomato genotyping. The highest percentage of polymorphism was obtained from primer ISSR6 and Sola 11 that is 100%, while the lowest percentage of polymorphism was from primer 10F. All of the ISSR primers gave a good percentage of polymorphism.

Polymorphism percentage was productive from some of ISSR primers were used in this study are same. We obtained some polymorphism (66.67%), in the primers (12F, 812, 889, B2, PHV6 and 825). Primer Sola 9 and Sola 4 produced 75% polymorphism and produced 83.33% polymorphism, respectively, from primer 13F and 16, but polymorphism to primer 12 and 816 was 80% and 87%, respectively.

The average polymorphic information content (PIC) value in fifteen ISSR primers was 0.50, ranged from 0.06 to 0.95 (Table 4.2), mean the PIC value was the lowest for ISSR primer PHV6 (0.06) and the highest value 10F (0.95), followed by B2 (0.89), 812 (0.86), while the value of PCI ranged from 0.06 to 0.64 and 0.09 to 0.67 respectively in the studies (Bredemeijer et al., 1998; He et al., 2003). Polymorphism information content value for each single ISSR primer was different, while primer 3F, Sola 5 and Sola 12 did not give any polymorphism information content value. The values of pair-wise genetic distances ranged from 0.1838 - 0.9049, indicating the attendance of extensive genetic diversity (Nadra et al., 2013).

The genetic matrix were divided to three groups (Figure 4.3). The dendogram have the most similar genotypes (G11-G17) had approximately 0.775 similarity indices.

The less similar genotypes (G15-G28, G9) had similarity indices from 0.390 to 0.641. García-Martínez et al., (2006) had close similarity indices among their tomato genotypes. According the classification groups in this study all genotypes of tomato were classified into three main groups. The first group was the smallest consisted of 1 genotype only that G15. The group two consisted the genotype 28. The third group was the largest and consisted of 30 different genotypes and classified into two main groups, each of them consisted sub main groups and each of them contained some similar branches respectively according Jaccard's similarity. Saida et al., (2013) had also obtained dendograms discrimination among tomato genotypes.

The highest similarity between 32 genotypes was 0.941, while the lowest was 0.261. Terzopoulos and Bebeli, (2008) found genetic similarity values ranged from 0.56 to 0.95 with an average of 0.797 among 41 tomato genotypes. The dendogram that showed all genotypes belong to the same group except G15 and G28.

ISSR markers gave high polymorphism rate and genetic variation was determined in tomato genotypes. Hence it was shown that the application of ISSR molecular marker technique is suitable for determining and analyzing genetic diversity among tomato genotypes collected from different geographical around Sulaymaniyah-Iraq Basin.

It was thought that analysis of these varieties will lead to the collection of information about the genetic diversity at the genome level also it is thought that this studied tomato gene pool will be an important genetic resource in breeding studies and will provide the convenience of germplasm management.

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Appendix 1. Jaccard similarity matrix.

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G20	G21	G22	G23	G24	G25	G26	G27	G28	G29	G30	G31	G32
G1	1.00																															
G2	0.69	1.00																														
G3	0.69	0.72	1.00																													
G4	0.67	0.81	0.79	1.00																												
G5	0.60	0.79	0.78	0.79	1.00																											
G6	0.74	0.71	0.88	0.77	0.74	1.00																										
G7	0.58	0.61	0.78	0.65	0.72	0.76	1.00																									
G8	0.59	0.61	0.74	0.63	0.60	0.63	0.73	1.00																								
G9	0.78	0.63	0.66	0.69	0.60	0.61	0.53	0.58	1.00																							
G10	0.67	0.74	0.79	0.79	0.76	0.84	0.81	0.76	0.66	1.00																						
G11	0.61	0.73	0.79	0.68	0.74	0.81	0.83	0.63	0.60	0.81	1.00																					
G12	0.72	0.68	0.77	0.71	0.68	0.68	0.69	0.71	0.78	0.79	0.75	1.00																				
G13	0.58	0.57	0.70	0.63	0.63	0.76	0.75	0.66	0.57	0.79	0.73	0.69	1.00																			
G14	0.56	0.59	0.66	0.55	0.59	0.74	0.73	0.59	0.52	0.72	0.86	0.71	0.73	1.00																		
G15	0.56	0.43	0.38	0.50	0.33	0.37	0.30	0.50	0.67	0.37	0.28	0.39	0.35	0.28	1.00																	
G16	0.60	0.67	0.82	0.69	0.78	0.83	0.94	0.69	0.56	0.84	0.83	0.72	0.72	0.75	0.29	1.00																
G17	0.70	0.83	0.82	0.79	0.81	0.81	0.72	0.72	0.67	0.90	0.82	0.84	0.76	0.74	0.35	0.79	1.00															
G18	0.56	0.73	0.74	0.66	0.77	0.79	0.82	0.61	0.54	0.81	0.89	0.73	0.79	0.80	0.30	0.84	0.81	1.00														
G19	0.68	0.73	0.80	0.74	0.68	0.76	0.69	0.74	0.74	0.86	0.77	0.87	0.72	0.68	0.47	0.72	0.88	0.73	1.00													
G20	0.64	0.68	0.78	0.66	0.70	0.83	0.82	0.63	0.63	0.83	0.93	0.73	0.79	0.83	0.30	0.82	0.81	0.85	0.80	1.00												
G21	0.64	0.59	0.71	0.69	0.58	0.73	0.63	0.63	0.66	0.76	0.65	0.76	0.63	0.57	0.50	0.65	0.73	0.60	0.83	0.70	1.00											
G22	0.67	0.69	0.80	0.65	0.75	0.78	0.81	0.66	0.65	0.79	0.88	0.74	0.69	0.79	0.30	0.81	0.84	0.78	0.81	0.86	0.69	1.00										
G23	0.57	0.66	0.71	0.65	0.68	0.78	0.75	0.62	0.55	0.80	0.84	0.66	0.73	0.83	0.30	0.76	0.78	0.82	0.71	0.83	0.70	0.80	1.00									
G24	0.64	0.63	0.78	0.68	0.71	0.71	0.76	0.56	0.65	0.68	0.81	0.69	0.65	0.71	0.26	0.76	0.73	0.69	0.70	0.79	0.64	0.81	0.75	1.00								
G25	0.65	0.71	0.84	0.80	0.71	0.93	0.73	0.70	0.66	0.85	0.77	0.75	0.75	0.68	0.35	0.81	0.83	0.77	0.82	0.79	0.71	0.75	0.70	0.70	1.00							
G26	0.63	0.65	0.79	0.64	0.70	0.81	0.80	0.60	0.61	0.78	0.89	0.68	0.72	0.78	0.26	0.80	0.79	0.80	0.77	0.88	0.69	0.91	0.79	0.82	0.80	1.00						
G27	0.59	0.64	0.75	0.65	0.70	0.81	0.80	0.62	0.60	0.83	0.91	0.72	0.83	0.83	0.29	0.85	0.81	0.89	0.77	0.93	0.67	0.84	0.88	0.79	0.79	0.85	1.00					
G28	0.57	0.50	0.56	0.56	0.57	0.50	0.65	0.48	0.56	0.52	0.53	0.50	0.63	0.54	0.44	0.57	0.50	0.54	0.52	0.55	0.40	0.59	0.45	0.57	0.54	0.57	0.55	1.00				
G29	0.59	0.69	0.73	0.67	0.72	0.75	0.78	0.63	0.60	0.81	0.92	0.72	0.75	0.85	0.29	0.80	0.83	0.86	0.79	0.89	0.63	0.85	0.87	0.81	0.74	0.85	0.93	0.53	1.00			
G30	0.61	0.65	0.76	0.66	0.75	0.72	0.81	0.60	0.62	0.75	0.88	0.78	0.69	0.85	0.27	0.83	0.80	0.80	0.75	0.85	0.63	0.88	0.78	0.87	0.71	0.83	0.87	0.57	0.91	1.00		
G31	0.60	0.59	0.71	0.65	0.64	0.76	0.72	0.54	0.61	0.73	0.86	0.67	0.75	0.80	0.33	0.75	0.75	0.81	0.73	0.87	0.63	0.80	0.79	0.79	0.75	0.84	0.92	0.62	0.92	0.86	1.00	
G32	0.69	0.73	0.78	0.80	0.69	0.69	0.65	0.73	0.74	0.83	0.75	0.84	0.70	0.70	0.44	0.70	0.85	0.69	0.88	0.75	0.72	0.74	0.74	0.75	0.75	0.69	0.78	0.52	0.80	0.77	0.72	1.00

CURRICULUM VITAE

I was born in Qaladze of Sulaimani - Iraq, in 1985. Have completed the primary education in Halsho Town and secondary education in Qaladze. During the years of 2007-2011 I had studied in Sulaimani University, the college of Agriculture and Department of Horticulture. In 2011 I had graduated from here. At the September of 2014 I started my master study in Yuzuncu Yil University.

EXTENDENT TURKISH ABSTRACT

SÜLEYMANİYE BÖLGESİNDEKİ DOMATES GENOTİPLERI ARASİNDAKİ GENETİK İLİŞKİLERİN SSRS MARKIRLARI İLE BELİRLENMESİ

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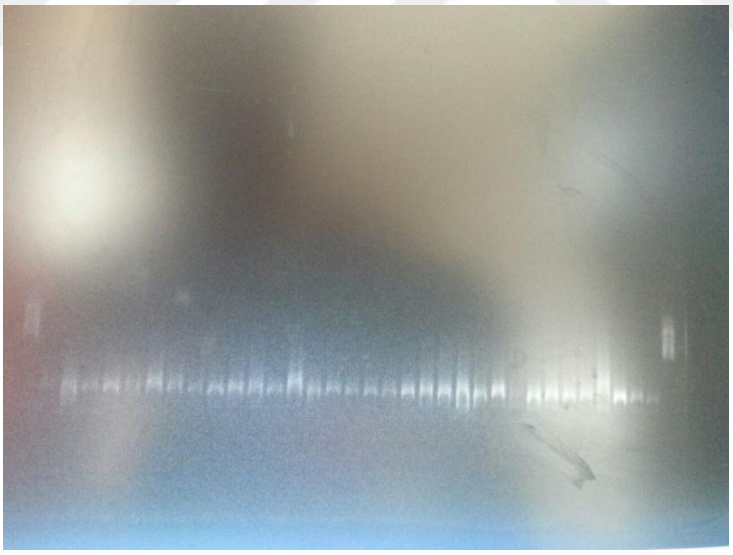
Bu çalışmada Irak-Süleymaniye bölgesinde yetiştirilen yerel domates genotiplerinin genetik ilişkilerini ISSR markır metodu ile analiz ederek genetik çeşitliliği ve genotiplerin birbirleri ile olan ilişkilerini ortaya konulması amaçlanmıştır.

Çalışmada kullanılacak olan genotiplerin tohumları iklim odasında 2:1 oranında karıştırılmış steril torf:perlit ortamına ekilmiştir. Çimlenen domates tohumları fide aşamasında gerçek yaprak oluşturduktan sonra DNA izolasyonu için yaprak örnekleri alınarak liyoflizatöre konulmuştur. İki gün boyunca -80 ºC sıcaklıkta ve düşük basınçta liyoflize edilen yapraklar öğütülerek izolasyon aşamasına geçilmiştir. DNA izolasyonu CTAB prosedürü kullanılarak yapılmıştır (Doyle ve Doyle, 1987). DNA miktarı ve kalitesi NanoDrop, ND 100 spektrofotometre ile belirlenmiş (Çizelge 1), ve final konsantrasyon 30 ng/µl olacak şekilde ayarlanarak -20°C'de muhafaza edilmiştir. İzolasyon için aşağıdaki adımlar takip edilmiştir:

1. Ezilen örnekler 2 ml'lik eppendorf tüplere konularak 700 µl CTAB tampon çözeltisi (100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, %2 CTAB, pH 8.0) eklenmiştir.
2. Tampon çözeltisi eklendikten sonar 10 µl ß-mercaptoethanol ve 10 µl proteinase eklenerek tüplerin ağzı kapatılmış, örnekler 65 ^{0}C sıcaklığa sahip su banyosu içerisinde 1 saat bırakılmış ve bu süre içerisinde birkaç kez hafifçe karıştırılmıştır.
3. Su banyosundan çıkarılan örnekler biraz soğuduktan sonra tampon çözelti ile eşit hacimde 24:1 hacimli kloroform:izoamil alkol konularak 13500 rpm'de 30 dakika santrifüj edilmiştir.

4. Santrifüj işlemi tamamlandıktan sonra ayrım tabakası oluşmuş ve bu tabakanın üstünde kalan sıvı yeni 2 ml'lik eppendorf tüplerine aktarılıp üzerine soğuk isopropanoldan 400 µl eklenip bir gün süreyle -20 ^{0}C'de bırakılmışlardır.
5. Bir gün sonra örnekler 13500 rpm'de 10 dakika santrifüj edilip DNA dibe çöktürülmüş ve geri kalan sıvı tüplerden uzaklaştırılmıştır.
6. Tüplere %76 etanol/10 mM amonyum asetat içeren çözeltiden 200 µl eklenerek 10000 rpm'de 5 dakika santrifüj yapılarak yıkanma sağlanmıştır. Bu işlem iki kez tekrarlanmıştır.
7. Yıkama tamamlandıktan sonra kurutma işlemi yapılarak 100 µl deiyonize su ve 5 µl RNAse eklenerek 37 ^{0}C'de bir saat inkubasyona bırakılmıştır. İnkübasyon süresi bittikten sonra izolasyon işlemi tamamlanarak DNA stok -20 ^{0}C'de muhafaza edilmiştir.

DNA miktarı ve kalitesi belirlendikten sonra DNA % 0.8 'lik agaroz jel üzerinde 90 V akımda 2 saat koşturularak UV altında görüntülenmiştir (Şekil 1).



Şekil 1. Genotiplerin %0.8 agaroz jeldeki DNA görüntüleri

PCR amplifikasyonu

25 ml amplifikasyon reaksiyonu; 2.5 µl 10X buffer, 2.5 µl MgCl2, 2 µl dNTP mix, her bir primerden 5 µl, 0.2 µl Taq DNA polymerase ve 5 µl olacak şekilde hazırlanmıştır. PCR kondüsyonu 94 °C'de 3 dakika 1 döngü, 94 °C'de 20 saniye,

primerin yapışma sıcaklığına göre 40 saniye ve 72 °C'de 1 dakika 33 döngü olacak şekilde yapılıp 72 °C'de 10 dakika ile PCR sonlandırılmıştır.

Çalışmadan Elde Edilen Bulgular

Genotiplerden izole edilen stok DNA'nın kalitesi ve miktarı Nanodrop cihazı kullanılarak belirlenmiş ve sonuçları Çizelge 4.1'de verilmiştir.

Çizelge 4.1. Domates genotiplerinin DNA miktarı ve kalitesi

Genotip	DNA miktarı (ng)	Kalitesi (A 260/280)
1	944.1	1.91
2	1077.1	1.67
3	994.7	2.02
4	920.1	1.89
5	787.7	1.73
6	1075.5	1.82
7	1008.3	1.88
8	1094.4	1.95
9	483.4	1.57
10	994.6	1.84
11	864.3	1.78
12	1438.8	1.80
13	704.2	1.76
14	1133.3	1.84
15	1550.4	1.87
16	605.9	2.00
17	601.9	1.60
18	1152.0	1.90
19	1125.4	1.77
20	1838.5	1.84
21	1031.0	1.74
22	1940.6	1.77
23	1561.4	1.90
24	1568.0	1.91
25	1631.4	1.58
26	680.9	2.00
27	1686.7	1.88
28	2019.0	1.95
29	1160.2	1.86
30	989.2	1.86
31	1210.4	1.71
32	968.9	1.73

Çalışmada kullanılan 18 ISSR primerinden 15'inden net okunabilen bantlar elde edilmiştir. Primerlerin polimorfizm oranları % 50 ile % 100 arasında değişmiştir. Her

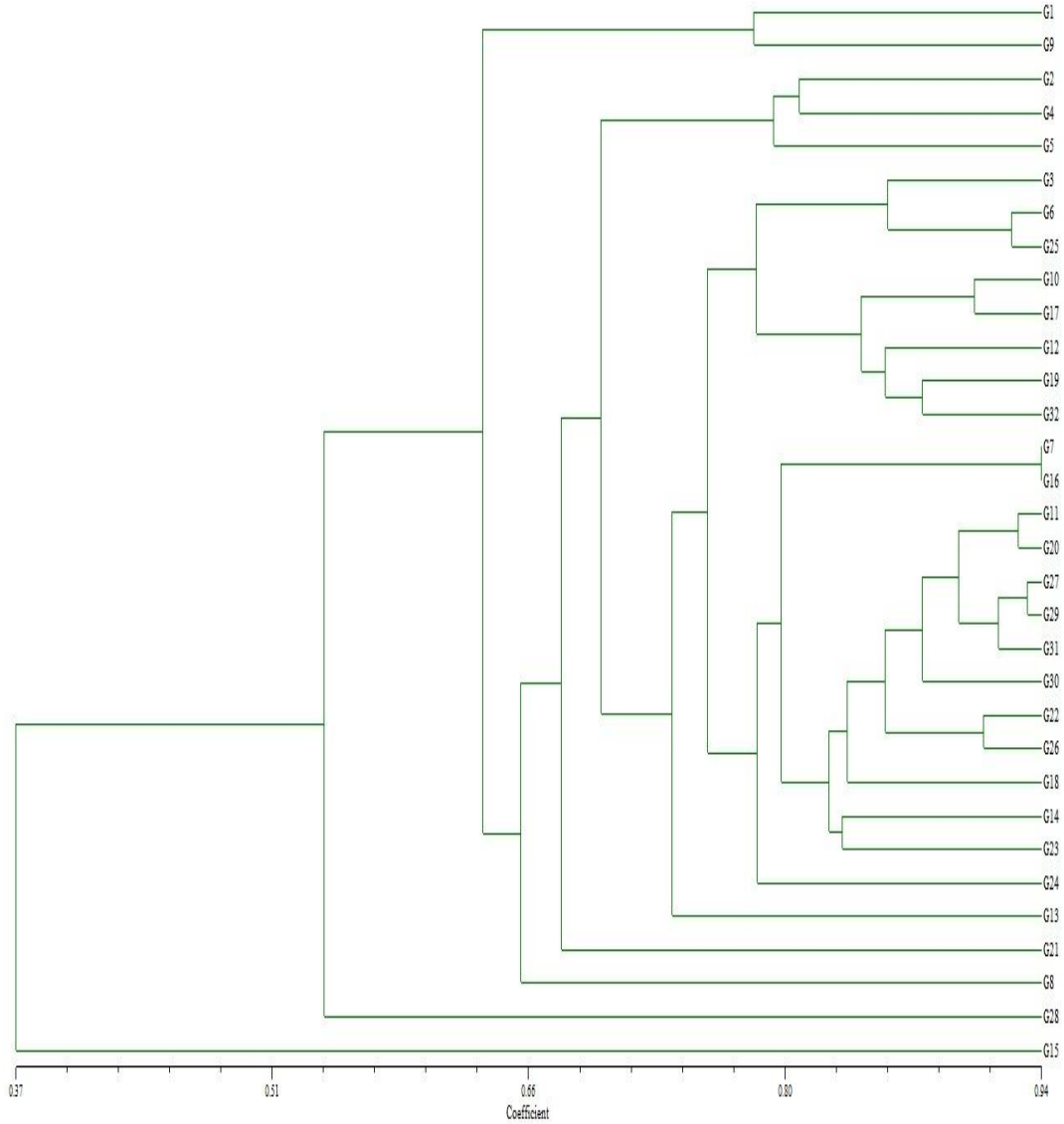
primer için PIC (Polimorfik bilgi içeriği) hesaplanmış ve en yüksek PIC değeri 0.95 ile 10 F primerinden elde edilmiştir. En düşük değerin ise 0.06 ile PHV6 primerinde olduğu tespit edilmiştir (Çİzelge 4.2).

Çizelge 4.2. Çalışmada kullanılan ISSR primerlerine ait bant sayıları ve PIC değerleri

No.	ISSR primerleri	Baz dizilimi (5'-3')	Toplam bant sayısı	Polimorfik bant sayısı	Polimorfizm (%)	PIC değeri
1	12F	$(AG)_8$YG	6	4	66.67	0.64
2	13F	$(AC)_8$YT	6	5	83.33	0.21
3	16	$(AC)_8$	6	5	83.33	0.20
4	812-B3	$(GA)_8$A	3	2	66.67	0.86
5	816	$(AC)_8$	8	7	87.50	0.61
6	889	AGTCGTAGT$(AC)_8$	6	4	66.67	0.64
7	B2	(AG)8T	3	2	66.67	0.89
8	ISSR6	VDV$(GT)_8$	4	4	100.00	0.34
9	PHV6	CCA$(CT)_8$	3	2	66.67	0.06
10	Sola 9	$(AC)_8$G	4	3	75.00	0.33
11	Sola11	GAG-$(CAA)_5$	2	2	100.00	0.25
12	10F-P4	$(GT)_8$YC	2	1	50.00	0.95
13	13	$(CCA)_5$	5	4	80.00	0.20
14	825	$(AC)_8$T	3	2	66.67	0.82
15	Sola 4	VHV-$(GT)_7$G	4	3	75.00	0.48
	Toplam		65	50		
	Ortalama		4.33	3.33	75.61	0.50

Jaccard benzerlik katsayısı kullanılarak elde edilen matrikse göre en farklı genotipler G15 (0.390), G28 (0.553), ve G9 (0.641) olurken, tüm genotipler arasında benzerliği en yüksek olanlar 0.775 benzerlik katsayısı ile G11, G17 ve 0.774 ile G20 genotipleri olmuştur. Genotipler arasında birbirine benzerliği en yüksek olanlar 0.941 benzerlik katsayısı ile G7-G16, 0.933 benzerlik katsayısı ile G27-G29 ve 0.930 benzerlik katsayısı ile G20-G27 genotip çiftleri olmuş. G15-G24 ve G15-G26 genotipleri ise 0.261 benzerlik katsayısı ile birbirine en uzak genotipler olarak tespit

edilmiştir. Benzerlik matriksi kullanılarak elde edilen dendograma göre genotipler temelde 3 gruba ayrılmıştır. İlk grupta G15 genotipi, ikinci grupta G28 genotipleri tek başına yer alırken, diğer genotipler ise üçüncü grupta konumlanmıştır (Şekil 4.5).



Şekil 4.4. Jaccard benzerlik matriksi kullanılarak elde edilen dendogram.