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Assessment of genetic variability among Jordanian tomato landrace using inter-simple sequence repeats markers

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Abstract

Twenty-nine Jordanian tomato landraces (*Solanum lycopersicum* L.) were characterized using inter-simple sequence repeats marker (ISSRs). Seven primers of ISSR could generate 77 markers; 51 of which were polymorphic. The lowest genetic similarity value (0.46) was found between landraces Jo964 and Jo955, while the highest (0.94) was obtained between landraces Jo983 and 29. The dendrogram shows that the samples are clustered in two main groups. The first group includes 4 landraces, and there are 25 landraces in the second group, which comprises one subgroup containing 13 landraces. Accessions collected from the Irbid region reported more mean values for the effective alleles (1.64), Shannon index (0.49), and heterozygosity (0.34). This study highlighted the diversity among Jordanian tomato landraces conserved in the National gene bank. These results will help in the establishment of a core collection for conserving collected landraces. The precise molecular characterization will help in efficient management and genetic improvement of local landraces.

Keywords:*Solanum lycopersicum*, landrace, ISSR, genetic diversity

1. Introduction

Tomato (*Solanum lycopersicum* L.) is an essential vegetable crop that is grown worldwide. The worldwide production of tomato and world area harvested was 182 million tonnes and 4.76 million hectares in 2018, respectively (FAOSTAT, 2018). Jordan produced 0.84 million tonnes in 2018. Because of it is economic importance as a food source, the tomato is considered one of the best genetically studied crops.

Tomatoes were probably domesticated for the first time in the Peruvian region or Mexico, according to De Candolle (1886) and Bauchet and Causse (2012), respectively. McCue (1952) reported that tomato was introduced first to Spain by Cortes and reached Italy through Naples, which was controlled by the Spanish at that time. From Europe, the tomato spread worldwide. During four centuries of cultivation, large numbers (7500) of landraces and varieties have been grown worldwide (Korir et al., 2014).

Landraces include local varieties, local populations, traditional cultivars, farmer varieties, and populations (Zeven, 1998), and traditional and primitive varieties (Negri *et al.*, 2009). Landrace are varieties with a high capacity to tolerate biotic and abiotic stress, resulting in high yield stability and an intermediate yield level under a low-input agricultural system (Zeven, 1998). In Jordan, a large number of tomato landraces were found. Since 1983, the National Agricultural Research Center collected seed samples of tomato landraces from local farmers throughout the country and maintained these samples in the seed bank (Qaryouti *et al.*, 2007).

Different DNA-based markers used to study the genetic variability among tomato varieties and landraces. These markers include random amplified polymorphic DNA (RAPD) (Salunke *et al.*, 2012, Foolad *et al.*, 1993), inter-simple sequence repeat polymorphic (ISSR) (Aguilera *et al.*, 2011, Sharifova *et al.*, 2017, Vargas-Ponce *et al.*, 2011), amplified fragment length polymorphism (AFLP) (Cebolla-Cornejo *et al.*, 2013, Ojha, 2003), simple sequence repeats (SSR) (Benor *et al.*, 2008, Saravanan *et al.*, 2014), and single nucleotide polymorphism (SNP) (Sim *et al.*, 2012, Cortés-Olmos *et al.*, 2015). The inter-simple sequence repeat markers in comparison with other molecular markers are powerful tools to assess the genetic diversity in tomato germplasm (Terzopoulos and Bebeli, 2008). ISSRs are simple, fast, and do not use radioactive materials. To the best of our knowledge, nothing has been done regarding the characterization of Jordanian tomato landraces using molecular markers. This work aims to study the genetic variability among 29 Jordanian tomato landraces using ISSR molecular marker.

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2. Materials and methods

2.1. Plant material

Twenty-nine Jordanian tomato landraces were investigated in this study. Seeds for landraces were obtained from the National Seed Bank belonging to the National Agricultural Research Center (NARC). Seeds were sown, and young and healthy leaves were harvested and stored at -20 °C until use. Figure 1 shows the collection sites of all landraces.

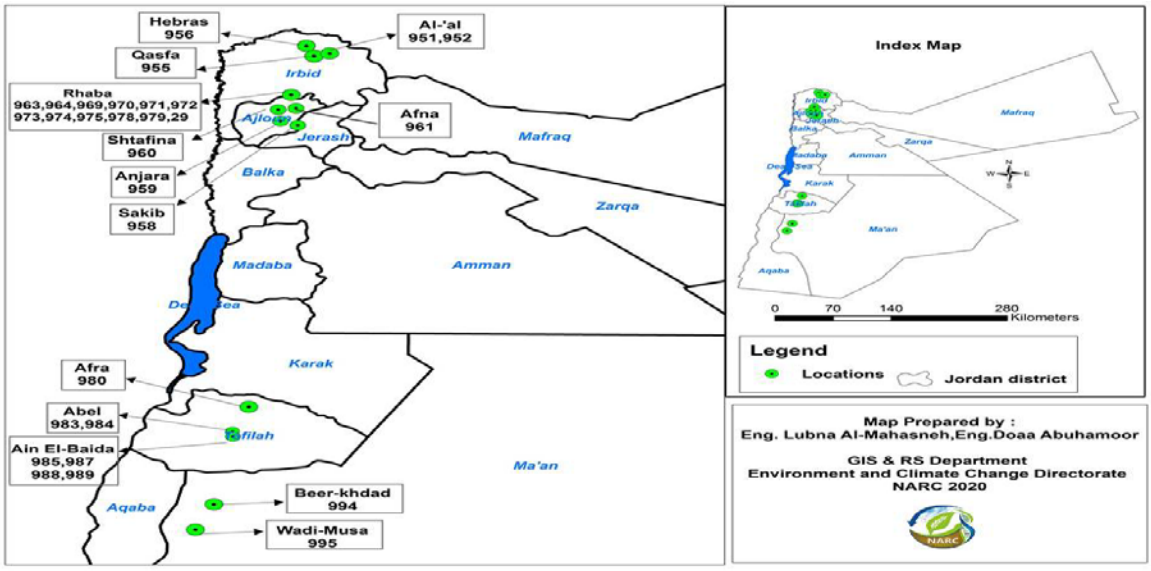

Figure 1. Map of Jordan showing collection sites of tomato landraces.

2.2. DNA extraction

Leaves were ground using a homogenizer. Total genomic DNA was extracted according to Doyle and Doyle (1987), the quality and quantity were determined using a spectrophotometer (NanoDrop 2000) and 1% agarose gel electrophoresis, respectively.

2.3. ISSR fingerprinting

Based on preliminary analysis, seven ISSR primers (Alpha DNA) (Table 1) were chosen and used for PCR amplification. According to Bornet and Branchard (2001), 25 µl reaction mixture containing 3µL of genomic DNA (10ng/µL), 0.3 µM of the primer, 1X Taq polymerase reaction buffer, 1.5 unit of Taq DNA polymerase and 0.2 mM of each dNTP were used in the amplification. Amplifications were performed using PTC-100 thermal cycler (MJ Research Inc., USA) programmed as follow, 5 min at 94 °C as an initial denaturation, followed by 40 cycles composed of 30 s at 94 °C, 45 s annealing at 52 °C, and 90 s at 72 °C and 72 °C for 5 min as a final extension. Using 1.5% agarose and 1X TBE buffer, the ISSR products were electrophoresed. Gene Ruler 100 bp DNA marker (ThermoFisher Scientific, USA) was used to estimate the fragment size and the gel stained using ethidium bromide (10 mg/ml), visualized by UV-transilluminator and photographed using an AlphaImager gel-documentation system according to Sambrook et al. (1989).

Table 1. Primers and their sequence, number of amplified bands, % prime efficiency, polymorphic markers, polymorphism, and discrimination power percentages.

Primer	Sequence 5'-3'	Total # of amplified bands	% primer fficiency	Polymorphic bands	% Polymorphism	% Discrimination power
UBC 807	$(AG)_8T$	12	16	11	92	22
UBC 809	$(AG)_8G$	15	19	11	73	22
UBC 810	$(GA)_8T$	11	14	1	9	2
UBC 811	$(GA)_8C$	12	16	11	92	22
UBC 817	$(CA)_8A$	6	8	4	67	8
UBC 823	$(TC)_8C$	11	14	8	73	16
UBC 825	$(AC)_8T$	10	13	13	50	10
Total		77		51		

2.4. Data analysis

For statistical analysis, the DNA profile for each landrace/primer was scored visually, and clear amplified bands were chosen. The presence of the band donated 1 and 0 for absence (Khierallah et al., 2014; Brake et al., 2014; Ng and Tan, 2015). Primer efficiency (the number of markers produced by primer/total number of markers obtained) was estimated and discrimination power according to Khierallah et al. (2011) for each primer was determined. The binary data collected by scoring ISSR profiles were used to calculate a similarity matrix using Jaccard's coefficients. Jaccard genetic similarity values were used to build the dendrogram based on the UPGMA method analyzed using NTSYSpc 2.02 software (Rohlf, 2000). Accessions were grouped into four main populations according to their origin. Irbid, Ajloun, Raba and Tafila with Ma'an. The total number of alleles, genetic diversity (He), and Shannon index for each population, and

the number of private alleles per population were calculated using GenAlex (Peakall and Smouse, 2012).

3. Results

The genetic diversity for 29 Jordanian tomato landraces was assessed in this study using molecular markers. Seven ISSR primers were used. Figure (2) is an example of the banding profile generated by ISSR primer UBC 823. The ISSR primers amplified 77 scorable markers (Table 1). The number of markers generated per primer varied from 6 (UBC 817) to 15 (UBC 809) and averaged 11 markers per primer. The size of the amplification products ranged from 200 to 1,900 bp. Fifty-one markers out of 77 were polymorphic with a percentage equal to 66% and an average of 7.3 polymorphic markers generated per primer. The primers UBC 807 and UBC 809 showed 11 polymorphic markers, while UBC 817 showed 4 polymorphic markers. The highest primer efficiency was obtained using the primer UBC 809, while the lowest obtained using the primer UBC 817 (Table 1). The highest value of discrimination power was observed using the primers UBC 807, UBC 809, and UBC 811, while the lowest was obtained by the primer UBC 810.

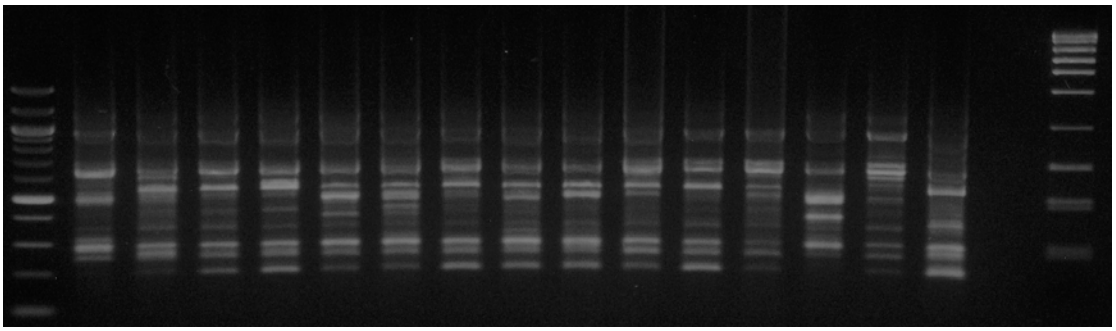

Figure 2. ISSR amplification profiles of 15 tomato landraces with primer UBC 823.

Based on Jaccard's genetic similarity coefficient, a dendrogram showing the relatedness between the landraces was constructed (figure 3). The lowest genetic similarity value (0.46) was found between landraces Jo964 and Jo955, while the highest (0.94) was obtained between landraces Jo983 and 29. The landraces are clustered into two subgroups, the first one comprises the landraces Jo964, Jo955, Jo969, and Jo78, and the second contains the other landraces. All landraces but one in the second subgroup are clustered in one main group with a genetic similarity value of 72.2%; this group is subdivided into further groups. At about 0.84 of genetic similarity, one interesting group contains 45% of the landraces (13) are formed and subdivided into two subgroups. The first contains 7 landraces, all but one from north Jordan and the second comprises 6 landraces, 4 of them from the southern part of Jordan.

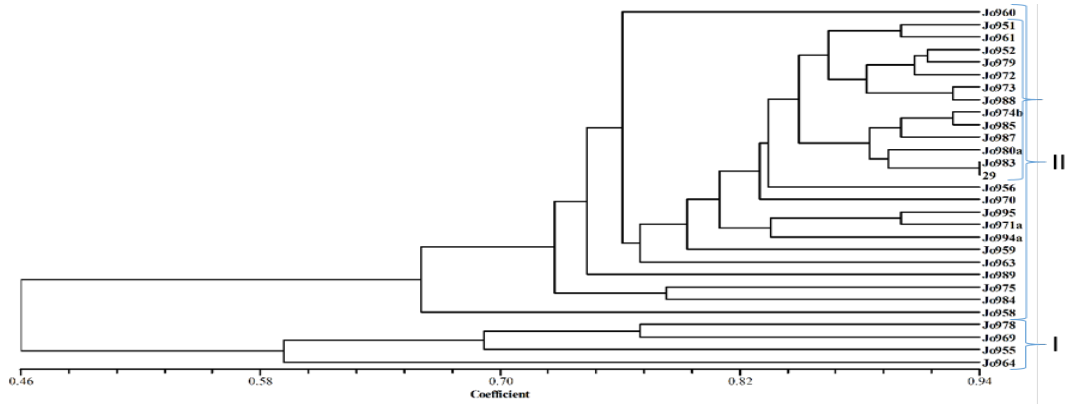

Figure 3. Dendrogram of 29 Jordanian tomato landraces generated by UPGMA cluster analysis of the genetic similarity values.

Table 2. Diversity parameters, of tomato landraces obtained from the analysis of ISSR alleles

Population	Number of samples	Na	Ne	I	H	%P	Private alleles
Irbid	4	1.64	1.64	0.49	0.34	76.62%	0.0
Ajloun	4	1.61	1.46	0.37	0.26	63.64%	0.0
Rhaba	12	1.75	1.61	0.48	0.33	80.52%	0.0
Tafila and Ma'an	9	1.52	1.38	0.32	0.22	57.14%	1.0
Mean		1.63	1.52	0.42	0.29	69.48%	

Na= Average No. of alleles, Ne = No. of effective alleles, I = Shannon index, H = diversity, P% percent of polymorphism. No. private alleles = No. of unique alleles for a single population.

The genetic diversity parameters analyzed among populations is present in Table (2). The average number of alleles (Na) varied between 1.52 for landraces collected from Tafila and Ma'an to 1.75 that collected from Rhaba. Accessions collected from the Irbid region reported more mean values for the effective alleles (1.64), Shannon index (0.49), heterozygosity (0.34). Tafila and Ma'an region showed 57.14% polymorphic percentage, and 80.52% was

reported for accessions collected from the Rhaba region. One private allele was recorded from the collection of Tafila and Ma'an.

4. Discussion

Molecular markers used in tomato including genetic variability, phylogenetic relationships, varietal fingerprinting, the marker-based selection, and the map-based cloning or QTLs (Ruiz et al., 2005, Sharifova et al., 2017, Kiani and Siahchehreh, 2018) .

In the last decades, the value of tomato landraces as gene source in many countries in the world has been explored (Cebolla-Cornejo *et al*., 2013, Corrado *et al*., 2014, Comlekcioglu *et al*., 2010); however, Jordanian tomato landraces were underrepresented. The evaluation of genetic variability is considered the first step toward effective utilization of crop genetic resources. In the presented work, molecular markers are used for the first time to evaluate the genetic variability among Jordanian tomato landraces.

The results reveal high percent of polymorphism (66%), which is consistent with results from previous analysis for tomato landraces using multilocus molecular markers in Turkey and Iran (Henareh et al., 2016, Kiani and Siahchehreh, 2018), Egypt (Hassan et al., 2013), Greece (Terzopoulos and Bebeli, 2008), Azerbaijan and other countries (Sharifova et al., 2017), Italy (Corrado et al., 2014), and Spain (Ruiz et al., 2005). Shahlaei et al. (2014) have used 10 ISSR primers to study genetic diversity for 10 tomato accessions and observed 23.25% of polymorphism percentage and 1.55 of an average resolving power value of markers was recorded. However, Mansour et al., (2010) reported high polymorphism in ISSR analysis (100%). In our study, polymorphism percentage ranged from 9 to 92% with an average polymorphic band of 7.3 per primer observed, which is more in other research. Edris et al., (2014) who reported a 62% polymorphism and a polymorphic band of 3.5 per primer. In contrary, 34% in Brazilian tomato cultivar using ISSR was reported (Aguilera et al., 2011).

The high polymorphism obtained could be explained by the nature of self-pollinating in tomato. Self-pollinating decrease intraspecific diversity while increasing interspecific diversity.

There is no clear clustering according to the collection site of landraces; however, some evidence was observed in the relationship with the site of collection. The first group in the dendrogram includes four landraces from north Jordan (Rhaba and Irbid), and the landraces Jo985, Jo987, Jo980a, and Jo983 which are collected from Tafila are clustered together. Furthermore, the landraces Jo961, Jo972, Jo973, and Jo979 are from closely related sites. This results are consistent with results of previous studies and explained that the accessions from different regions might have the similar genetic background and those from the same origin might also have different genetic background (Keneni et al., 2005; Gashaw et al., 2007; Celka et al., 2010; Comlekcioglu et al., 2010, Aguilera et al., 2011; Sharifova et al., 2013 and 2017) and in disagreement with other studies (Terzopoulos and Bebeli, 2008). Factors behind increasing the number of alleles, and increased gene diversity, varied among genetic diversity studies could be different molecular markers uses, i.e. dominant vs codominant markers, resolution of marker alleles using different electrophoresis system in fragmentation of amplified products and size and geographic composition of the tested germplasm. The presented study confirms that ISSR molecular markers are powerful tools to assess genetic diversity among Jordanian tomato landraces.

5. Conclusion

This study investigated the genetic variability of 29 Jordanian tomato landraces was investigated using ISSR molecular-based DNA marker. Nevertheless, using different types and more polymorphic molecular markers (SSRs, SNPs) could be necessary to distinguish the accessions with unknown origin and biological status. These results will help in the establishment of core collection and better conservation of Jordanian tomato landraces. The DNA-based characterization will help in efficient management and maintenance in the genetic improvement approaches. Furthermore, evaluation of these landraces under abiotic stress could be useful to produce new tomato varieties coping with climate change.

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