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Molecular characterization of some tomato genotypes using inter simple sequence repeats (ISSR) technique

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Abstract
The local tomato genotypes have many important traits comparing to the introduced hybrids. Theses genotypes are considered as valuable genetic resources that should be preserved, so in this study, the genetic variability was evaluated for 11 of the most important local genotypes comparing to 4 wild species. The ISSR technique was used with 21 primers which amplified 194 bands, including 172 polymorphic bands with a polymorphism average of 80.915%. The number of total bands per primer varied from 2 to 20 with an average of 10.777. The average of PIC and GD values were 0.4919 and 0.5151, respectively. The cluster analysis based on genetic diversity between studied genotypes showed that the studied genotypes were divided into 5 groups, each group included one of the 4 wild species and the fifth one included all local genotypes. This study proved that the ISSR technique was succeeded in distinguishing between different tomato genotypes on molecular level.

Keywords: **Molecular characterization, Tomato, ISSR, Genotypes, Wild species.**

Introduction
Tomato (*Lycopersicom esculentum*) is considered one of the most widespread vegetable crops in the world and of economic importance (Ali and colleagues, 2011). Tomato is ranked second after potato (*Solanum tuberosum*) with a global production of 162 million tons, and a cultivated area of 7 million hectares (FAOSTAT, 2012). On the local level, Syria ranked nineteenth in the world and sixth in the Arab world in tomato production, which reached 783,874 tons, with an estimated cultivated area of 14579 hectares (FAOSTAT, 2012).
Many traditional methods of evaluating and characterizing the different genotypes of tomatoes have been used, as most of the local tomato genotypes have been described based on the morphology and productivity. The classification of plant species depending on the morphological and production characteristics are one of the common and widely used methods, However, many of these characteristics are often influenced by the prevailing environmental conditions, which may give different results that are difficult to rely on distinguishing the variations (Claros et al, 2000; Wjhani, 2004).), as it also requires time and effort, so it has become necessary to support it with modern biotechnology methods, the most important of which are molecular markers that are accurate and fast in distinguishing between genetic structures, they also require a small amount of DNA (Ricciardi et al, 2002). These methods are based on the study of the molecular markers of DNA, which are characterized by their wide numbers and not affected by environmental conditions, and can be used in determining genetic diversity and estimating genetic similarity (Eleuch et al, 2008).
Inter Simple Sequence Repeats (ISSR) is one of the most used techniques to the study of genetic variation in plants. It has been used in genetic studies of many plant species as it is effective in detecting very low levels of genetic variation.
In tomatoes, ISSR technique was used to assess the genetic variation between 33 local varieties of Greek tomato genotypes and three hybrids of cherry tomato (*S. lycopersicum var. Cerasiforme*), and two genotypes of *Solanum pimpinellifolium* (Terzopoulos and Bebeli, 2008). ISSR technique was also used to assess the genetic variation between 96 tomato genotypes in Brazil (Aguilera et al, 2011). In another study, ISSR technique was used to study the genetic relationship between 5 wild species of tomato (*Lycopersicom esculentum, Lycopersicom pennellii,*

Lycopersicom cheesmanii, Lycopersicom humboldtii, and *Lycopersicom hirsutum*) and two of the nine strains of *Lycopersicomulentum*. Nine out of fourteen used primers were able to distinguish among the studied species (Tikunov et al., 2003). Tomato cultivation in Syria is based on many enhanced local genotypes, in addition to the introduced foreign varieties and hybrids. The local genotypes of tomatoes are distinguished by many important characteristics compared to the introduced hybrids, such as good adaptation to local environmental conditions, their resistance to various biological and abiotic stresses, and their high content of important nutrients such as vitamins and minerals, thus these models are considered as a valuable genetic stock that provides an excellent genetic base for genetic improvement of tomato and hybrid production at the local level. In spite of the good specifications of these models, and due to the superiority of the introduced hybrids in terms of production, the use of local genotypes is neglected, and their spread began to be limited to only a few areas and on a narrow level, which could expose them to extinct. Therefore, this study aims to carry out the molecular characterization of some locally genotypes of tomato and to study the genetic similarity among them in comparison with some known wild species.

Materials and Methods

Tissue culture and molecular analyzes were carried out at the laboratories of the Public Authority for Biotechnology in Damascus, and at the Vegetable Research Laboratory at the Faculty of Agriculture - University of Damascus during the period 2014-2015.

Plant material: In this study, four wild species of tomato were studied: *Lycopersicum cheesmanii, Lycopersicum pimpinellifolium, Lycopersicum chilens* and Solanum *pennelli* obtained from the International Gene bank of the *Solanaceae* (https://solgenomics.net). In addition to eleven of the most important locally grown tomato genotypes, which are characterized by high productivity and quality, which are: Barouk, Qanawat, Harran, Rihani, Jalin, Wedian Al Rabeea, Doumani, Nola Sikka, Karazi, Jabali and Masyaf their seeds were obtained from Dr. Bassam Abu Turabi, local markets, farmers and the Public Authority for Biotechnology. We propagated them using tissue culture technology in order to obtain healthy plants free of contamination, and to obtain a sufficient amount of vegetative growth for use in research.

Molecular characterization:

DNA extraction: Isolation of DNA from 0.2 g of healthy leaves, taken from plants of 30 days old by the modified CTAB method (Murray & Thompson, 1980). To each sample, 750 µl of extraction solution was added, which contains:

(2% (w / v) CTAB, 100 mM Tris-HCl (pH 8), 1.4 ml NaCl, 20 mM EDTA (pH8), 0.2% 2-mercaptoethanol (v / v)).

Then we put it in a water bath at a temperature of 60°C for 60 minutes. Then 750 µl of chloroform / isoamyl alchohol (1: 24) was added to each sample, mixed well, and then centrifuged at 12,000 rpm for 10 minutes at 4 ° C. The upper phase was then transferred to new tubes and added two-thirds of its volume isopropanol, which was cooled at -20°C, and the samples were then left at -20 ° C overnight in order to precipitate the DNA. On the next day, centrifugation was done at 10,000 cycles / min for 5 minutes at 4 ° C, then the filtrate was removed and the sediment was washed with 1 ml of 70% ethanol. The tubes were then centrifuged at 10,000 cycles / min for 5 minutes at 4 ° C, ethanol was excluded, the DNA dried at room temperature for 30 minutes, and dissolved in 100 µl of sterile distilled water and kept at -20 ° C.

The DNA quality was visualized using 1% agarose gel electrophoresis method, containing 2 µl of ethidium bromide at a concentration of 10 ml / mg in TBE (1X) solution consisting of 89 mM of Tris, 89 mM of boric acid, 2 Mm of EDTA (PH 8), in horizontal electrophoresis at 100 Volts. The DNA concentration was also measured using a UV spectrophotometer, and then the DNA concentrations in all samples were standardized to 50 ng / µl.

DNA replication: DNA was amplified using 21 Inter Simple Sequential Repeats (ISSR) primers (Table 1) by a thermal cycler with a total reaction volume of 25 µl (12.5 µl of Go Taq Green Master Mix, 8.5 µl of sterile distilled water, 10 pmol of primer, 100 ng/l of DNA), according to the following conditions:

• The first stage (the first denaturation stage): It takes place once at 95 ° C for 5 minutes.

• The second stage: repeated 40 times and includes the following steps:

- Denaturation: The two strands of DNA were separated from each other at a temperature of 95 ° C for one minute.
- Annealing: The Primer bind of to the target DNA at the appropriate temperature according to the used primer for one minute and as shown in Table (1).
- Extension: The new strands DNA are completed at a temperature of 72°C for one minute.

• The third stage: It takes place once at 72°C for 5 minutes to end all reactions.

Separation and photograph of PCR products: The amplification products were separated in the electrophoresis on a 2% agarose gel containing 2 µl of ethidium bromide at a concentration of 10 ml / mg, in the presence of a 100 bp molecular weight indicator (KAPA Universal Ladder, KAPA BIOSYSTEMS). And photographed in the presence of ultraviolet radiation (Figure1)

Statistical analysis: The size of the DNA bands was determined using TotalLab (Ultra Lum Inc., Claremont, Calif.) Software. Then, the number of bands (total, different, similar) was estimated, the data converted to the binary system (1 for the existing band and 0 for the absent band). Then, a genetic incompatibility matrix was calculated based on Jaccard's coefficient, and then this matrix was used to perform Unweighted Pair Group Method of Arithmetic Means (UPGMA) and draw the genetic tree using Power Marker software. Also, the genetic diversity rate (percentage of polymorphism) and the Polymorphism Information Content (PIC) were calculated for each used primer at the single locus level (Weir, 1996) according to the following relationship:

PIC = 1- Σ pi2

pi: the frequency of each allele on the same locus.

Gene diversity (GD) was also calculated for each of the primer within the studied group of genotypes (Nei, 1987) according to the following relationship:

(n-1) / GD = n (1-Σ pi2)

n: number of entries and p is the percentage of occurrence of each allele on the same locus.

Results and Discussion

The DNA of the studied tomato genotypes was amplified by using 21 primers of Inter Simple Sequences Repeats (ISSR) (Table 1), 19 of them gave amplification results, and each of the P8, P12 and P20 primers did not give any amplification, so they were not included in the results. The total number of bands from DNA amplification of the studied genotypes was 194, including 172 polymorphic bands with a polymorphism average of 80.915%. The number of total bands per primer varied from 2 to 20 with an average of 10.777 (Table 2). The primers P3, P6, and P7 gave the highest number of total bands (18, 18 and 20, respectively), while the primer P4 gave the lowest number of total bands (2 bands). As for the number of different primers, it ranged between 0 and 20 bands, with an average of 9,555 bands per primer (Table 2). P7 gave the largest number of varied bands (20 bands) with 100% polymorphism, as well as both P3 and P6 gave a large number of varied bands (18 bands) with 100% polymorphism. Both P11 and P14 gave 100% polymorphism but with fewer varied bands (9 and 6 bands, respectively), while P4 did not give any differentiated bands with 0% polymorphism. These results are consistent with the findings of Edris and colleagues (2014), as the results of this study indicated that the total number of the bands reached 63. Of these, 39 bands varied with a polymorphism of 62%, and the average number of bands for each primer was 5.7, as P3, P11 and P18 gave the highest polymorphism (75, 80 and 67%, respectively), while P13 and P21 each gave the lowest polymorphism (50 and 25%, respectively).

The polymorphism information content (PIC) of a genotype, thus a specific molecular marker, represents the ability of this marker to distinguish between genotypes. The average polymorphism content of the used primers in this study was 0.4919, with the highest value being 0.7631 for the primer P17, while its lowest value was 0.1167 for the primer P4 (Table 2). The primers that are most capable of distinguishing between genotypes were the ones that gave the highest value of polymorphisms information content (PIC) which are: P17, P7, P5, P11, P13 (0.7631, 0.7206, 0.6771, 0.6516, and 0.6372, respectively). The results of this study contrasted with the results of a study of the genetic diversity of eight species using the ISSR technique. The average value of the PIC was 0.36, while the value of the PIC when using the AFLP technique was 0.37 (Edris et al., 2014). In another study of the genetic diversity of ten strains of tomato using ten ISSR primers, the PIC values ranged between 0.051 and 0, with a mean of 0.088, and the highest PIC value when using the (GA) 8A initiator was about 0.234, while the initiator (AG) 8G gave the lowest value. For PIC (0.051) (Torabi et al., 2014). The results of the study of ten varieties of tomato indicated that the best primer was K-13, with a PIC of 0.858 (Sanghani & Mandavia, 2013).

In addition to the PIC values, the gene diversity (GD) values were calculated for each of the used primers in this study. The gene diversity values indicate the extent of genetic diversity among the studied genotypes. The results of this research showed that there is a large variation in the GD values between the used primers, as its value ranged between 0.1244 for the P4 primer and 0.7911 for the P17 primer, with an average of 0.5151 (Table 2). GD values have been used in many studies to indicate the level of genetic diversity among the studied genotypes. In a study to assess genetic diversity on 21 genotypes of tomatoes in Pakistan using 20 RAPD primers, the highest value of gene diversity for the GL A-12 primer was about 0.99 The lowest value was about 0.12 for the GL A-09 primer, with an average of 0.4 (Rasheed et al,. 2015). In another study assessing the genetic diversity among eight genotypes of Bulgarian tomato using SSR technique, the mean GD value was 0.222 (Radkova et al,. 2015). It should be noted that the used primers in this study, which gave the highest value of polymorphism information content (PIC) are the same that gave the highest value for gene diversity (GD), and the value of the content of polymorphism (PIC) for each primer was close to the value of gene diversity (GD) of the same primer (Table 2).

Determining the genetic similarity among the genotypes is very important for plant breeding programs to secure a wide genetic database to be used in crossbreeding programs. In this study, the genetic similarity among the studied tomato genotypes was determined by calculating the genetic variation matrix, which showed the value of the genetic variation among the studied genotypes, and the high value of this variation indicates an increase in genetic similarity among the genotypes (Table 3). Cluster analysis of the studied genotypes was also performed based on the genetic variation matrix, as the cluster analysis allows drawing the genetic similarity tree and dividing the studied genotypes into groups, and these groups reflect the degree of genetic similarity among them, and may be grouped into one group based on their original habitat. According to the cluster analysis of the studied genotypes in this study, it was noticed that they were divided into five different groups, where the wild species were separated into independent groups, while all the local types were grouped into one group (Figure 2), and this can be explained by the fact that all these genotypes have the same wild origin which is cherry tomatoes *Lycopersicum esculentum* var. *cerasiforme* that was moved from South America to Mexico where it was domesticated and then had spread to the rest of the world (Harvey et al., 2002). The first group included the wild type *L. cheesmanii*, and it was associated with the rest of the groups at the highest hierarchical level with a genetic variation level of 0.735. It was observed that the wild species *L. chilense* was the closest genetically to *L. cheesmanii* with a genetic variation of 0.710, while the wild species *L. pimpinellifolium* and *S. pennelli* were the farthest genetically from it with a genetic variation of 0.759. The second group included the wild type *S. pennelli,* and it was associated with the rest of the groups at the level of 0.674, and the lowest degree of genetic similarity between *S. pennelli* and the wild type *L. cheesmanii* with a genetic variation of 0.759, while the highest degree of genetic similarity was between *S. pennelli* and the Baroque type with a genetic variation of 0.662. (Figure 2; Table 3). As for the third group, it included the wild type *L. chilense* and it was associated with the rest of the groups at a genetic variation level of 0.458, and the lowest degree of genetic similarity between *L. chilense* and the wild type was *L. cheesmanii* with a genetic variation of 0.710, while the highest degree of genetic similarity between *L. chilense* and the type Rihani With a genetic variation of 0.440. The fourth group included the wild type *L. pimpinellifolium* and this group correlated with the fifth group at a genetic variation level of 0.428, the greatest genetic variation between *L. pimpinellifolium* and the wild type L. cheesmanii (0.759), while the lowest genetic variation was with the Rihanian type 0.413 (Fig. 2; Table 2) 3).

The fifth group included 11 genotypes: Barouk, Wedian Al Rabeea, Qanawat, Masyaf, Doumani, Noula El Sekka, Glenn, Harran, Rihani, Jabali and Karazi. The genetic similarity differed between these types, as it was divided into two groups: the first subgroup included one genotype, the Baroque genotype, which was the farthest genetically from the wild type, *L. cheesmanii*, with a genetic variation of 0.734, while the two genotypes were genetically close to Baroque, Harran and Glenn, with genetic variation value of 0.163, while the second subgroup included the rest of the genotypes with a genetic variation of 0.078, and it was divided under this group into two sub-sub clusters: the first sub-sub cluster, included only Wedian Al Rabeea, and the highest degree of genetic similarity for it with the genotype Rihani with genetic variation 0.052 while the lowest genetic similarity with the wild type, *L. cheesmanii*, with a genetic variation of 0.727, the second sub-sub cluster, the rest of the genotypes that were in turn subdivided into two sub-sub-sub clusters were included at a genetic variation level of 0.067. the first sub-sub-sub clusters subdivided, at a genetic variation level of 0.0411, into three groups: the first group, comprising the two genotypes of Mesiaf and Kanawat, and the wild type *L. cheesmanii* was the farthest genetically from the genotype of Kanawat with genetic variation of 0.727, while the two genotypes, Harran and Domani, were the closest Transgenic of the two genotypes Mesiaf and Kanawat (Figure 2; Table 3). The second group included the two genotypes Nola Sekka and Domani, and the genetic variation between them was about 0.0204. The third group was divided into two parts, the first part included Glenn at a genetic variation level of 0.025, and the Harran model was the closest genetically to it with a genetic variation of 0.021, while the second part included Rehani and Harran, and the genetic variation between them was 0.01, as Kanawat genotype was the genetic closest to the genotype Harran with a genetic variation of 0.021. As for the sub-subgroup of the second group, it included both the karazi and the jabali genotypes, with a genetic variation between them about 0.059, and the highest degree of genetic similarity between the karazi and Nola sikka was with a genetic variation of 0.051, and between the Jabali and the Nola sikka with a genetic variation of 0.049 (Figure 2; Table 3).

The previous results indicate the possibility of using the ISSR technique to study the genetic similarity among the local tomato genotypes and the known wild tomato genotypes. The ISSR technique has been used previously in many studies to assess the genetic variation and study the genetic similarity between tomato genotypes. This technique was used to assess the genetic variation between 33 local varieties of Greek tomato (*Solanum lycopersicum*) and three hybrids of cherry tomato (*S. lycopersicum var. cerasiforme*), and two genotypes of *Solanum pimpinellifolium*. Using 12 primers, all of them gave formal plurality between the studied models, with an average genetic similarity of 0.797, and these types were divided into two main groups: the first included local varieties, while the second included cherry tomato hybrids and *S. pimpinellifolium* (Terzopoulos and Bebel 2008). These results are consistent with what was found in this study, where all the local genotypes were clustered in one group separate from the groups of the wild species. In another study in which the genetic similarity between 5 genotypes of tomato (*L. esculentum, L. pennellii, L. cheesmanii, L. humboldtii and L. hirsutum*) and two strains of *L. esculentum* was estimated using the ISSR technique, using 14 primers, Nine of them were able to distinguish between all the studied genotypes, and *L. esculentum, L. humboldtii* and *L. cheesmanii* were observed to separate into one group, while *L. hirsutum* and *L. pennellii* were in a different group (Tikunov et al, 2003).

Table 1. Primers used for DNA amplification of the studied tomato genotypes

Primer code	Primer name	Primer sequence	Annealing temperature (^oC)
P1	SLISSR 1	(CCA)5	50
P2	SLISSR 2	GAG(CAA)5	40
P3	814	(CT)8TG	40
P4	844A	(CT)8AC	35
P5	844B	(CT)8GC	40
P6	17899B	(CA)6GG	35
P7	17899A	(CA)6AG	35
P8	SLISSR 8	(GTG)3GC	35
P9	SLISSR 9	(AG)8	35
P10	HB13	(GAG)3GC	35
P11	17898B	(CA)6GT	35
P12	SLISSR 12	(CAC)5	45
P13	HB14	(CTC)3GC	35
P14	SLISSR 14	(GA)8GG	35
P15	SLISSR 15	(AG)8TG	40
P16	SLISSR 16	(ATG)5	35
P17	SLISSR 17	(GCC)5	60
P18	17898A	(CA)6AC	35
P19	SLISSR 19	CTG(AG)8	40
P20	SLISSR 20	(AC)8	35
P21	HB12	(CAC)3GC	35

Table 2. Number of bands (total, dissimilar, similar), proportion of polymorphisms (%), polymorphism content, gene variation

Primer code	Bands number	Polymorphic bands number	Common bands number	Percentage of polymorphism (%)	PIC	GD
P1	7	4	3	57.143	0.3295	0.3467
P2	10	6	4	60	0.3295	0.3467
P3	18	18	0	100	0.5132	0.5333
P4	2	0	2	0	0.1167	0.1244
P5	13	11	2	84.615	0.6771	0.7111
P6	18	18	0	100	0.4251	0.4444
P7	20	20	0	100	0.7206	0.7556
P9	16	15	1	93.75	0.4251	0.4444
P10	5	4	1	80	0.4962	0.5244
P11	9	9	0	100	0.6516	0.6756
P13	4	3	1	75	0.6372	0.6667
P14	6	6	0	100	0.4962	0.5244
P15	10	9	1	90	0.5132	0.5333
P16	6	3	3	50	0.3092	0.3378
P17	9	8	1	88.8899	0.7631	0.7911
P18	10	9	1	90	0.4251	0.4444
P19	16	15	1	93.75	0.5132	0.5333
P21	15	14	1	93.333	0.5132	0.5333
Total	194	172	22			
Average	10.777	9.555	1.222	80.915	10.777	0.5151

Table3. Matrix of the genetic variation between the studied tomato genotypes based on Jaccard's coefficient

Studied genotypes	*L. cheesmanii*	*L. pimpinellifolium*	*L. chilense*	*S. pennelli*	Barouk	Qanawat	Harran	Rihani	jalin	Wedian Al Rabeea	Doumani	Noula El Sekka	Karazi	Jabali	Masyaf
L. cheesmanii	0														
L. pimpinellifolium	0.759	0													
L. chilense	0.710	0.474	0												
S. pennelli	0.759	0.709	0.679	0											
Barouk	0.734	0.438	0.488	0.662	0										
Qanawat	0.727	0.449	0.476	0.674	0.200	0									
Harran	0.732	0.421	0.448	0.669	0.163	0.042	0								
Rihani	0.734	0.413	0.440	0.672	0.171	0.052	0.010	0							
Jalin	0.732	0.433	0.460	0.679	0.163	0.062	0.021	0.031	0						
Wedian Al Rabeea	0.727	0.437	0.464	0.664	0.217	0.102	0.062	0.052	0.082	0					
Doumani	0.736	0.430	0.457	0.664	0.196	0.041	0.041	0.051	0.061	0.081	0				
Noula El Sekka	0.736	0.417	0.444	0.664	0.179	0.061	0.021	0.031	0.041	0.061	0.020	0			
Karazi	0.734	0.425	0.452	0.682	0.189	0.071	0.051	0.061	0.051	0.091	0.070	0.051	0		
Jabali	0.746	0.415	0.442	0.676	0.216	0.107	0.069	0.059	0.069	0.088	0.068	0.049	0.059	0	
Masyaf	0.732	0.433	0.460	0.669	0.181	0.021	0.021	0.031	0.041	0.082	0.021	0.041	0.071	0.087	0

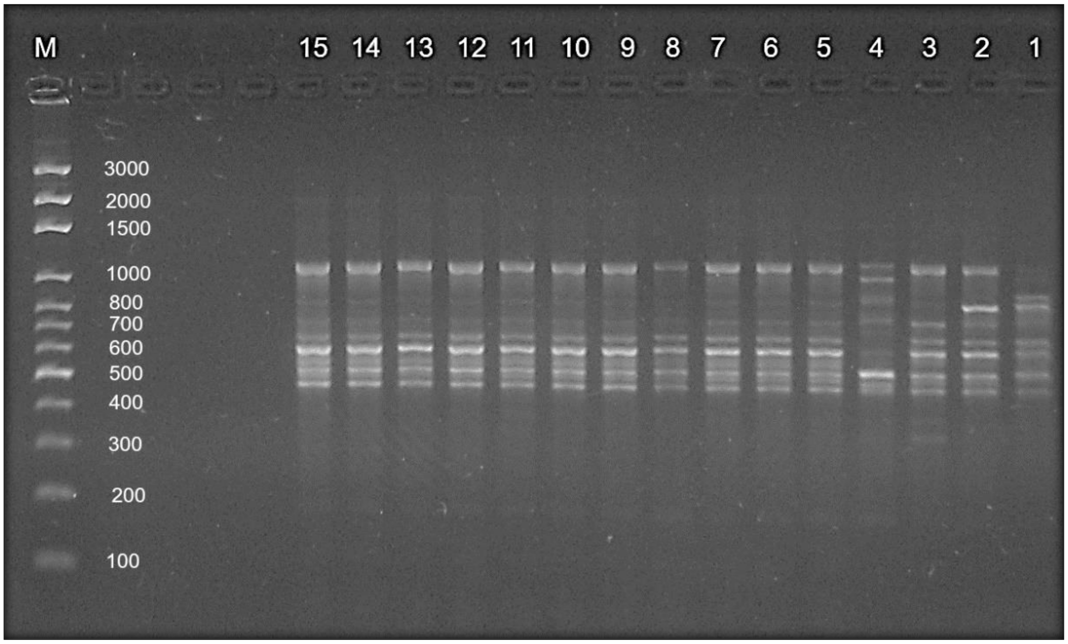

Figure 1. An image of the agarose gel of the DNA amplification products of the studied tomato genotypes using the P2 primer

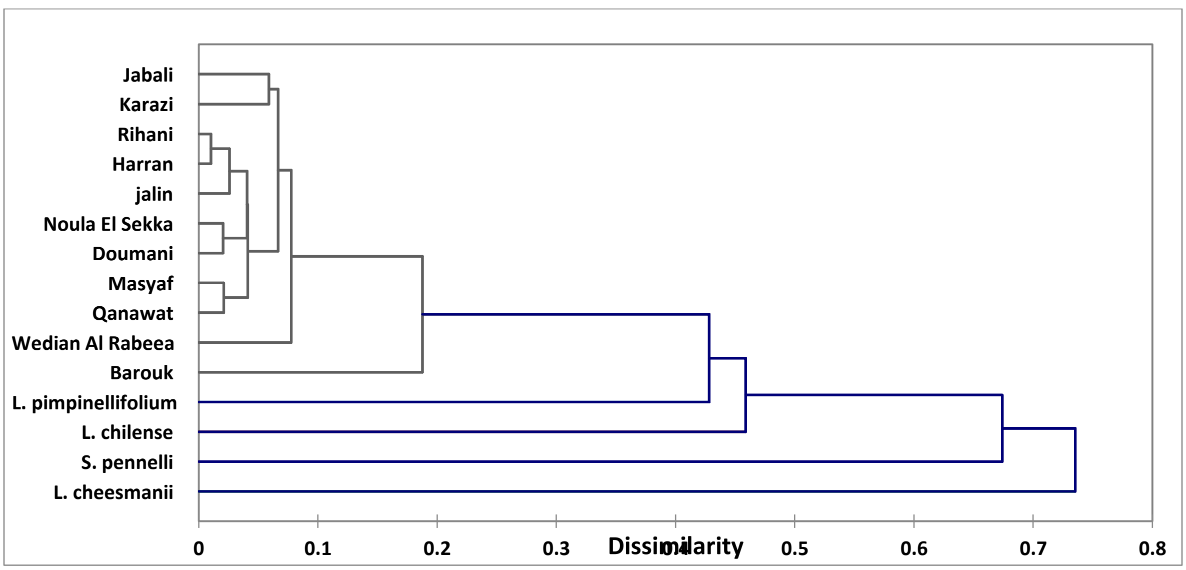

Figure 2. **Dendrogram of genetic relationships between the studied tomato genotypes using the UPGMA method.**

The ISSR technique was also used to study the genetic diversity of 54 genotypes of tomatoes for several species of the genus *Lycopersicum*, where it was observed that one species, *L. hirsutum var. glabratum* was clearly separated from the rest of the genus, while the other species formed two groups: the first included *L. pimpinellifolium*, *L. cheesmanii* and many of the genotypes of *L. esculentum*, while the second group included the genotypes of the other species (Kochieva et al, 2002).

This study demonstrated the success of the ISSR technique, in particular the used primers, which gave a high polymorphism, in distinguishing between local genotypes of tomatoes and the studied wild species at the genetic level. This study indicates the extent of the diversity and importance of the local genetic resources of tomato, which can be used in the various breeding and genetic improvement programs to obtain hybrids and varieties of tomato with high adaptation capacity while maintaining production efficiency. Therefore, it is recommended to use the ISSR technique and the used primers in this study to distinguish between other locally grown tomato genotypes and to relation the morphological characterization with the molecular characterization of the studied genotypes.

References

Aguilera, J.G., L. A. Pesson, G. B. Rodrigues, A. Y. Elsayed, D. J. H. da Silva and E. G. De Barros. 2011. Genetic variability by ISSR markers in tomato (*Solanum Lycopersicom* Mill.). Revista Brasileira de Ciências Agrárias. 2: 243-252.

Ali, S. G., N. Rab, N. Khan and K. Nawab. 2011. Enhanced proline synthesis may determine resistance to salt stress in tomato cultivars. Pakistan Journal of Biological Sciences. 43(6): 2707-2710.

Claros, G.M., R. Crespillo, M.I. Agilar and F.M. Canovas. 2000. DNA fingerprinting and classification of geographically related genotypes of olive tree (*Olea europaea* L.). Euphytica. 116:131-142.

Edris, S., S. Abo-Aba, M. M. Algandaby, A. M. Ramadan, N. O. Gadalla, M. A. Al-Kordy, J. S. M. Sabir, F. M. El-Domyati, A. M. Alzohairy and A. Bahieldin. 2014. Molecular characterization of tomato cultivarsgrown in Saudi Arabia and differing in earliness of fruit development as revealed by AFLP and ISSR. Life Science Journal. 11(8):602-612.

Eleuch, L., A. Jalil, S. Grando, S. Ceccarelli, M.K. Schmising, A. Tsujimoto, A. Daaloul and M. Baum. 2008. Genetic Diversity and association analysis for salinity tolerance, heading date and plant height of barley gemoplasm using simple sequence repeat markers. J. Integr. Plant Biolo. 50(8): 1005-1015.

FAOSTAT, Food and agriculture organization of the United Nations. *2012*. http://faostat.fao.org/.

Harvey, M., Quilley, S. and Beynon, H. 2002. Exploring the Tomato. Transformations of Nature, Society and Economy. Edgar Publishing, Cheltenham, UK, 304 pp.

Kochieva, E. Z., N. N. Ryzhova, I. A. Khrapalova and V. A. Pukhalskyi. 2002. Genetic Diversity and Phylogenetic Relationships in the Genus *Lycopersicom* (Tourn.) Mill. as Revealed by Inter-Simple Sequence Repeat (ISSR) Analysis. Russian Journal of Genetics. 38(8): 958–966*.*

Murray, M.G. and W. F. Thompson. 1980. Rapid isolation of high molecular weight DNA. Nucleic Acids Res. 8: 4321- 4325.

Nei, M. (1987). Molecular Evolutionary Genetics. Columbia University Press, New York, NY.

Ricciardi, L., V. Giorgio, C. DeGiovanni, C. Lotti, A. Gallotta and G. Fanizza. 2002. The genetic of apulian apricot genotypes (*Prunus armeniaca* L.) assessed using AFLP markers. Cellular and Molecular Biology Letters. 7: 431-436.

Sanghani, AO and MK, Mandavia. 2013. Characterization of Tomato (*Lycopersicom lycopersicum* Mill.) Genotypes through RAPD, ISSR and SSR Markers. Indian Journal of Agricultural Biochemistry. 26(2): 141-147.

Rasheed, A., M. A. Shah, A. I. Khan, F. S. Awan, H. A. Sadaqat, S. Bahadur, and F. S. Baloch. 2015. Genetic Diversity of Some Tomato Cultivars and Breeding Lines Commonly Used in Pakistani Breeding Program. Turkish Journal of Agriculture - Food Science and Technology. 3(3): 126-132.

Torabi, S., A. Shahlaei and M. Khosroshahli. 2014. Efficiacy of SCoT and ISSR marekers in assesment of tomato (*Lycopersicum esculentum* Mill.) genetic diversity. International Journal of Biosciences. 5(2): 14-22.

Terzopoulos, P. J and P. J. Bebeli. 2008. DNA and morphological diversity of selected Greek tomato (*Solanum lycopersicum* L.) landraces. Scientia Horticulturae. 4: 354–361.

Tikunov Yu, M., L. I. Khrustaleva and G.I. Karlov. 2003. Application of ISSR markers in the genus *Lycopersicom*. Euphytica. 1: 71-81.

Radkova, M., E. Todorovska, A. Ivanova, D. Ganeva, G. Pevicharova, E. Molle, B. Bojinov and Z. Danailov. 2014. Biotechnology & Biotechnological Equipment Assessment of genetic variation in Bulgarian tomato (*Solanum lycopersicum* L.) genotypes, using fluorescent SSR genotyping platform. Biotechnology & Biotechnological Equipment. 28(1): 68–76.

Weir, B. S. 1996. Genetic data analysis II 2nd ed Sinauer Associates. Inc. Sunderland, MA.

Wjhani, Y. 2004. Genetic studies on the biodiversity of local and wild Syrian wheat using modern biotechnological techniques. Thesis of doctor, Department of genetics, Cairo University, Faculity of agriculture. 119 p.