التوصيف الجزيئي لحشرة ذبابة التبغ البيضاء (*Bemisia tabaci* Genn.) المنتشرة في بيئات الساحل السوري باستخدام المؤشر الجزيئي ISSR

Molecular Characterization of *Bemisia tabaci* Genn. Spread in Syrian Coastal Environments Using ISSR Markers

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الملخّص

تصنف ذبابة التبغ البيضاء *Bemisia tabaci* Genn. ضمن الأنواع الغازية عالمياً وفقاً للاتحاد الدولي لحفظ الطبيعة والموارد. هدفت هذه الدراسة إلى تحديد التباينات الوراثية لمجتمعات حشرة ذبابة التبغ البيضاء المنتشرة على نباتي البندورة والباذنجان في الساحل السوري، وذلك باستخدام تقانة ISSR ومقارنة النتائج بنتائج سابقة استخدمت فيها تقانة RAPD. بلغ عدد الحزم الناتجة 102، ونسبة التعددية الشكلية 0.75. في حين كان التباين بين المجتمعات المدروسة 14.3 %، ونسبة التشابه 77.5 %. لوحظ وجود علاقة ارتباط ضعيفة بين العائل (البندورة والباذنجان) ومجتمعات الذبابة ($r = 0.04$). بينت شجرة القرابة توزع المجتمعات في مجموعتين تبعاً للارتفاع عن مستوى سطح البحر $r = 0.04$ وإلى ثلاث فئات حسب بيئتها الزراعية. وكان الارتباط ضعيفاً بين تقانة ISSR وRAPD وفق اختبار Mantel ($r = 0.2$). أكدت هذه الدراسة كفاءة تقانة ISSR في الكشف عن التنوع الوراثي لذبابة التبغ البيضاء.

الكلمات المفتاحية: ISSR، RAPD، ذبابة التبغ البيضاء، البندورة، الباذنجان، سورية.

Abstract

According to the International Union for the Conservation of Nature and Natural Resources (IUCN) whitefly *Bemisia tabaci* Genn. classified in the globally invasive species. This study was aimed to characterize this insect molecularly by detecting the genetic variations of its populations on tomato and eggplant in the Syrian coast region. So, ISSR markers were used to compare the results with these obtained by RAPD. The results showed that the number of ISSR bands was 102 with 0.75 polymorphism, the variance among populations was 14.3 % and the similarity was 77.5 %. Our results also showed a weak correlation between the host (tomato and eggplant) and the insect populations ($r = 0.04$). The dendrogram was divided into two principal groups depending on the heights of sea level and distributed in three classes according to the agricultural environment. A weak correlation was also found between RAPD and ISSR markers using Mantel test $r = 0.2$. This study reinforce the efficiency of ISSR marker in detecting the genetic diversity of whitefly *Bemisia tabaci* Genn. populations.

Keywords: ISSR, RAPD, Whitefly, Tomato, Eggplant, Syria.

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Introduction

According to the International Union for the Conservation of Nature and Natural Resources (IUCN) whitefly *Bemisia tabaci* Genn. was classified in the globally invasive species. It causes economic damages to a wide range of crops in the tropical, subtropical and moderate areas (Brown, 1994; Brown and Bird, 1992). This species was divided into many populations, that differ in biological characteristics (Bedford, *et al*., 1994; Costa and Brown, 1991; Stansly and Naranjo, 2010). This pest frequently attacks tomato (*Lycopersicon esculentum* L.) and eggplant (*Solanum melongena* L.) that considered as economic crops in Syria (Annual Statistical, 2011). Tomato planting in Autumn has been stopped in cause of a critical infection by Tomato Yellow Leaf Curl Virus (TYLCV), which is transmitted by B. tabaci. (Abboud, 2007). Whitefly, B. tabaci, is a complex species that include of at least 34 different biotypes, which are morphologically indistinguishable, but differ genetically from each other (Boykin, *et al*., 2007; Boykin *et al*., 2012; Dinsdale *et al*., 2010).

Molecular markers were considered as benefit tools to identify biotypes that could be used in integrated pest management programs and environmental preservation. Random Amplified Polymorphic DNA polymerase chain reaction (RAPD-PCR) (Gawell and Bartlett, 1993; Williams *et al*., 1990) and Inter Simple Sequence Repeats (ISSR) (Dong *et al*., 2008), 9were used to study the genetic diversity and evolution of populations (Esselman *et al*., 1999; Hess *et al*., 2000; Wolfe and Kephart. 1998).

This study aimed to: a) retracethe geneic variation accuracy among the populations of whitefly on tomato and eggplant on the Syrian coast using ISSR markers, b) determine the correlation between samples in both hosts and environmental conditions, and c) compare the results obtained with those obtained using RAPD markers in same samples (Mouhanna and Barhoum, 2014).

Materials and Methods

Sample collection

19 Samples of Whitefly *B. tabaci* Genn. were collected from mid-May to September, in greenhouses and fields at sea level up to 1200 meters in the Syrian coast region (Lattakia, Jabbleh, Banias and Tartous) (Tabel 1). Each sample insects were reared separately for eight generations on eggplant, and replaced regularly by a new plant during for rearing period. Eggplants were covered by plastic cages (10 x 10 x 30 cm) provided with vents, and these vents were covered by soft gaze, under greenhouse condition (25 ± 2ºC, relative humidity 65 – 70%, and photoperiod 16:8 h) at Biological Control Studies and Research Center – Damascus University (Syria).

DNA Extraction

Total DNA was extracted from 20 individual of each insects sample at the end of the eighth generation and suspended in distilled water, according to a Cetyl Trimethyl Ammonium Bromide (CTAB) protocol (Drayton, *et al*., 2009), with several modifications such as using proteinase K 20 ng/µl in the extraction buffer 2% CTAB, 100 mM Tris-cl pH 8.0, 20 mM EDTA, 1.4 M NaCl. The quality of extracted DNA was checked using spectrophotometer at the ratio 260 and 280 nm wavelengths. The DNA concentration was adjusted at 10 ng/ml, and the samples were stored at -20 °C.

ISSR-PCR

Six ISSR primers were used $(GACAC)_3$, $(GACA)_4$, $(TCC)_5$, $(AGG)_5$, $(ACTG)_4$ (Perring, 1993) and (5'-AGAGGTGGGCAGGTG-3') (Gong *et al*., 2001) (Table 2). Amplification reactions were done in a 25 µl volume containing 12.5 µl PCR master kit (Promega), 6.5 µl free nuclease distilled water, 4 µL (40 ng) template DNA and 2 µl primer of each (10 pmol/µl). PCR were done on a thermal cycler (peqSTAR 96 Universal Gradient), as follows: an initial denaturing step of 5 min, at 94 °C, followed by 35 cycles of 1 min denaturing at 95 °C, 1.5 min. annealing at 50 °C, 2 min. extension at 72 °C, and

Tabele 1. Locations and hosts of the collected samples

Region	Location	Altitude (m)	Host	Agricultural practice	Code
Lattakia	Around Slenfa	900	eggplant	field*	Eg.18
	Snober	<100	eggplant	greenhouse	Eg.7
	Around Qurdaha	600	eggplant	field*	Eg.14
	AinElbeda	<400	eggplant	field	Eg.1
	Elbasa	<50	tomato	greenhouse	To.8
	Elbahlolia	600	tomato	field*	To.16
Jableh	RaasElain	<50	eggplant	field	Eg.6
	Beat yashot	850	tomato	field*	To.17
	Zheriat	<50	tomato	greenhouse	To.13
Banias	Marqp	370	eggplant	field	Eg.3
	NabiaElsin	<100	tomato	greenhouse	To.12
	Around Mosfat	<50	tomato	greenhouse	To.11
Tartous	Dabosia	<100	eggplant	field	Eg.5
	Miar Shaker	<200	eggplant	field	Eg.4
	Karto	<100	eggplant	field	Eg.2
	Around Drakish	800	tomato	field*	To.19
	Tnakho (Elshaar)	1200	tomato	field*	To.15
	Around Qadmos	250	tomato	greenhouse	To.10
	Karto	<100	tomato	greenhouse	To.9

*** Locations at 400- 1200 meters above sea level.**

a 5 min. final extension at 72 °C. PCR products were analyzed by gel electrophoresis in 1.5 % agarose in 1x TRIS-Borat-EDTA (TBE) buffer. Gels were stained with ethidium bromide (8µl/100ml of TBE) and digitally photographed under ultraviolet light in a transilluminator documentation system.

Statistical Analysis

Genetic similarity between whitefly samples was performed using POPGEN32. The amplified bands were scored manually as 1 (present) and 0 (absent), depending on Jaccard coefficient (Rohlf, 1993). Clustering dendrogram was constructed by un-weighted pair group method using arithmetic average (UPGMA) method using PopGene program (Yeh *et al*., 1997). XLSTAT program was used to estimate the variation between the Clustering classes dendrogram and the influence of hosts in genetic diversity (Addinsoft, 2014), Mantel test was used to measure correlation coefficient r that determines the relationship between similarity index matrices (Mantel, 1967).

Results and Discussion

Polymorphism

All used primers appeared to be binded to one or more sites of whitefly's genome. The results of electrophoresis revealed the 102 amplified fragments, 77 of those were polymorphic, with polymorphism percentage of 75 % and an average of 12.8 fragment/primer (Table 2). The range of polymorphism percentage among samples was between 55 % (GACAC)3 and 100% $(ACTG)_4$. The highest number of amplified fragments was obtained by $(AGG)_5$ (26 Fragments). The number of amplified fragments differed among samples, and ranged between 78 bands on To.16 (Al-Bahloleah-Lattakia) and 56 on Eg.14 (Qardaha-Lattakia) (Data not showen).

Moreover the primer $(GACA)_4$ revealed a genetic variation between samples, where the total number of bands was seven. The number and molecular weight of bands were compared between the samples, and was found that the samples (Eg.6, To.15, To.16, To.17, To.19), (Eg.1, Eg.2, Eg.3, Eg.4), (Eg.7, To.8, To.12, To.13, To.18) and (To.9, To.10, To.11) had the same bands number three, five, six and seven respectively and the same molecular weight. Whereas the sample Eg.14 (Qardaha - Lattakia) showed only one band and Eg.5 seven bands, one of them was distinct and its size was 400 pb (Fig.1). Such results revealed that the percentage of polymorphism was 82.31 % using RAPD markers thatapplied on the same samples using 11 primers (Mouhanna and Barhoum, 2014).

Table 2. Number of DNA bands generated by ISSR primer used in this study.

Primer sequence	Total number of DNA fragments	Polymorphic Bands	Percentage of polymorphism%
$(GACAC)_3$	20	11	55
$(GACA)_4$	7	6	85.7
$(TCC)_5$	14	10	71.42
$(AGG)_5$	26	18	69.23
$(ACTG)_4$	15	15	100
5'AGAGGTGGGCAGGT3'	20	17	85
Total	102	77	75
Average	17	12.83	77,725

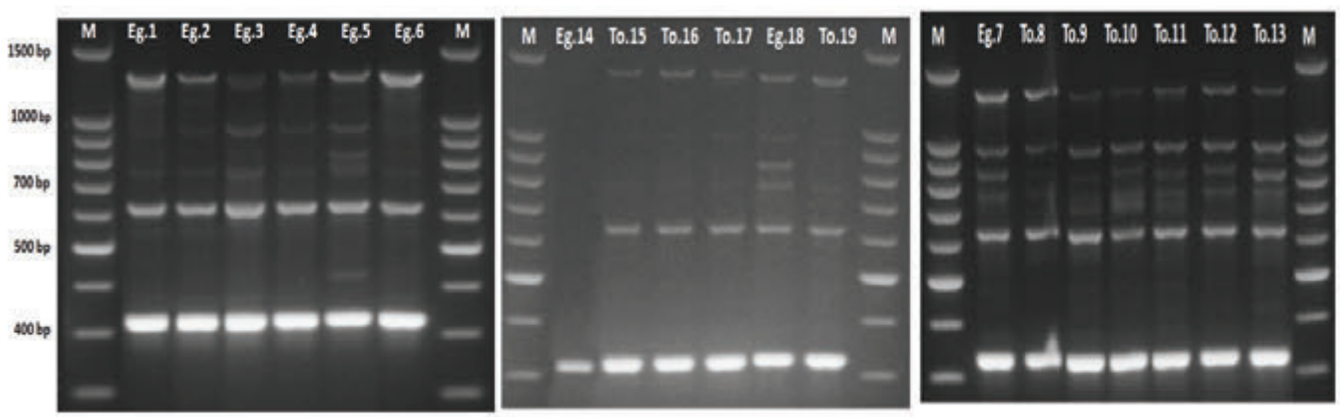

Figure 1. Results of ISSR-PCR products using primer $(GACA)_4$ on 1.5% agarose gel. M: molecular weight DNA marker (100 bp), Eg: Insects collected from eggplant, To: Insects collected from tomato.

Cluster analysis and genetic relationships

Cluster analysis was achieved at three levels as follows:

The first level, included samples from eggplant showing a phylogenetic tree divided into two clusters (Fig. 2) at the percentage of disagreement values (PDV) of 0.60. The 1st group (Group1) represented about 66.7 % of all samples and contained six samples from fields' range of altitude between 400 - 1200 m. A 2nd group (Group 2) containing three samples from greenhouse and fields' altitude below 400 m represented 33.3 %. The genetic similarity was ranged between 89 % (Eg.18, Eg.2) and 57 % (Eg.4, Eg.6) (Table 3) and the variation among samples was 11.7 %.

According to the results obtained RAPD-PCR in same samples from eggplant showed a phylogenetic tree was divided into two clusters and the percent disagreement values (PDV) was 0.47. The first group contained eight samples Eg.1, Eg.2, Eg.3, Eg.4, Eg.5, Eg.6 and Eg.7 while the second contained Eg.14 and Eg.18. The variation range among samples was 18.59 % (Mouhanna and Barhoum, 2014).

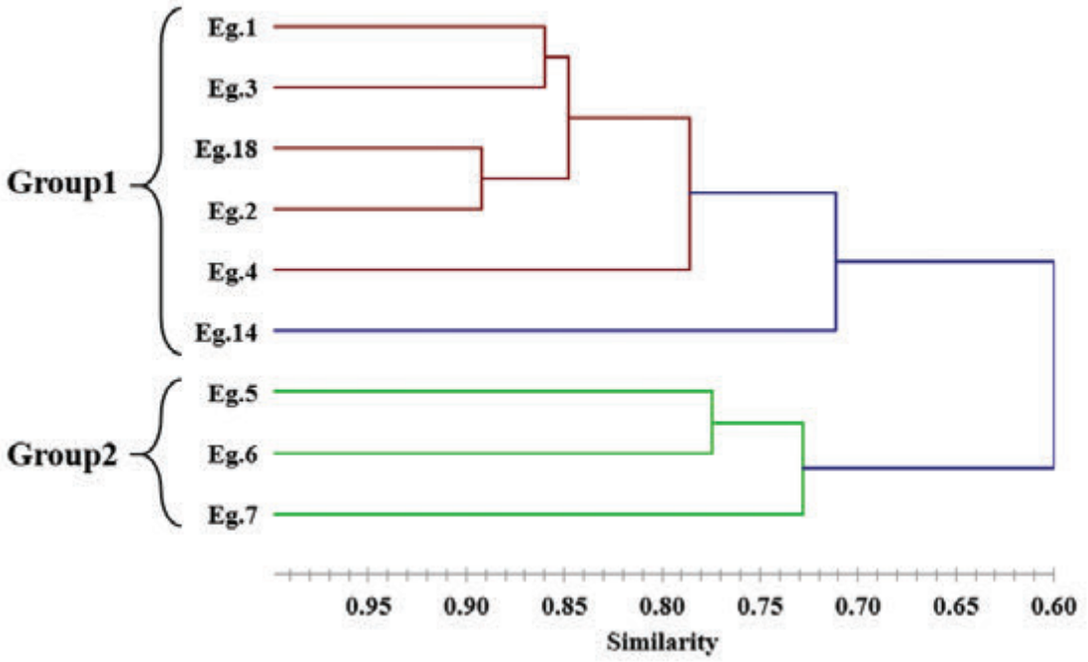

Figure 2. Cluster analysis of *B.tabaci* samples on Eggplant from sea level to a height of 1200 m according to Jaccard coefficient / UPGMA method.

The second level, including samples from tomato showed a phylogenetic tree divided into two clusters (Fig. 3) at the percent disagreement values (PDV) of 0.64. The **Group A** represented about 30% of all samples and contained three samples of fields' altitude more than 400 m. Whereas **Group B** contained seven samples from greenhouse and only one (To.19) from altitude about 800m and represented 70 %. The genetic similarity was ranged between 86 % (To.15, To.16) and 60 % (To.15, To.13) (Table 3) and the variation among samples was 10.9 %. According to the results of RAPD-PCR in same samples, showed a phylogenetic tree divided into two clusters at the percent disagreement values (PDV) 0.5 except the To.19. The first cluster included To.8, To.9, To.10, To.11, To.12 and To.13 samples, whereas the the second contained To.15, To.16, To.17 and To.19 samples. Variation range among samples was 24.9 % (Mouhanna and Barhoum 2014). The distribution of To.19 differed from the rest, could be explained by its source that belong to low-height areas (>400 m), where the tomato seedlings were cultivated and processed by farmers for commercial purposes, therefore, some immature insects are probably moved with seedlings during their transfer to other places.

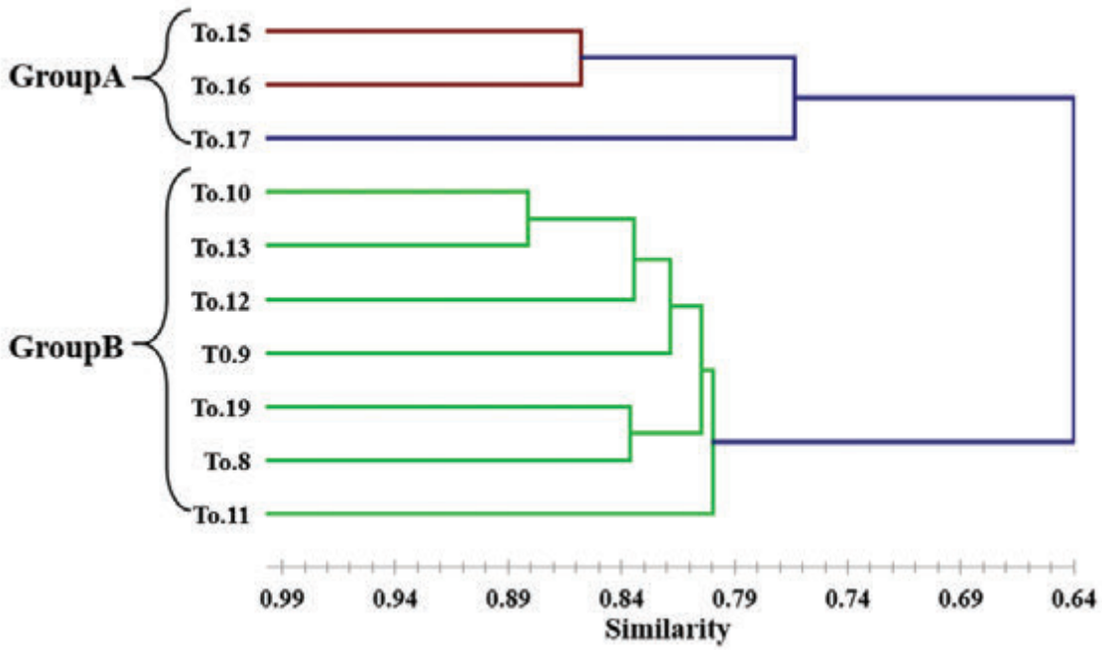

Figure 3. Cluster analysis of *B.tabaci* samples spread on Tomato from sea level to a height of 1200 m according to Jaccard coefficient/ UPGMA method.

The third level including the study of similarity among all samples and the effect of location and the height above sea level. The phylogenetic tree was divided into two main groups (G1, G2) at the value 0.57 of PDV (Fig. 4). G1 divided into two subgroups, subG.1 contained the samples from both greenhouses and open fields (up to 1200 m) with 31.5 % of representation, whereas subG.2 included all samples from greenhouses excepting To.19 from open fields (800 m), that represented about 37%. In contrary, G2 contained samples from open fields (from sea level up to 1200 m), with 31.5 % of representation. The variation between samples was 14.3 %.

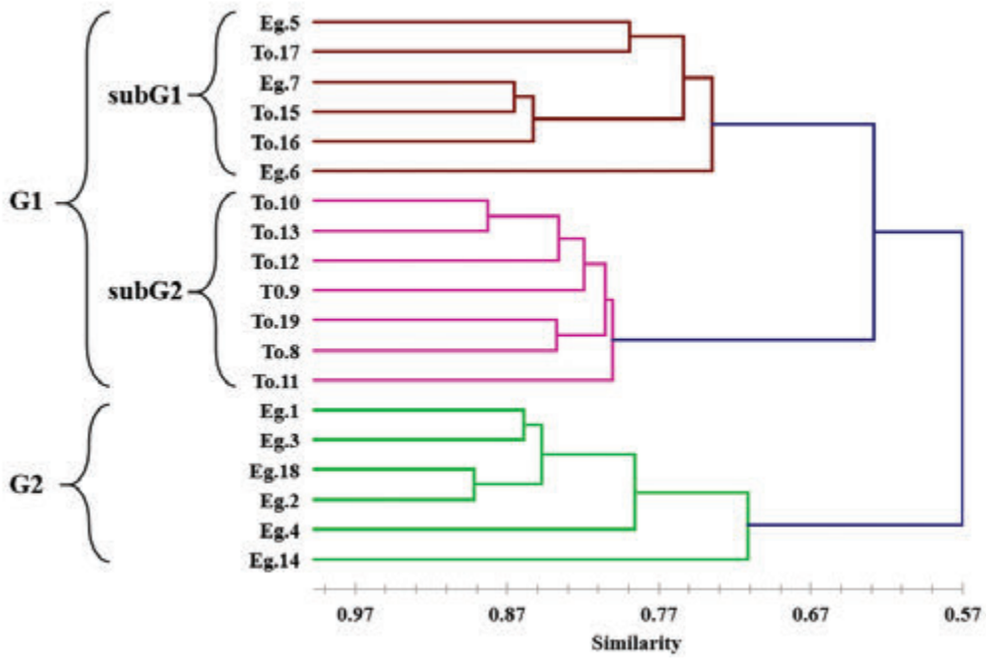

Figure 4. Cluster analysis of *B.tabaci* samples on Tomato and Eggplant from the sea level to a height of 1200 m using on ISSR markers according to Jaccard coefficient/UPGMA method, G: main group, SubG: Subgroup.

Tabele 3. The Jaccard coefficient and the percentage of genetic similarity between the samples of whitefly using ISSR markers.

Sa	To.13	To.12	To.11	To.10	To.9	To.8	To.19	To.17	To.16	To.15	Eg.7	Eg.6	Eg.5	Eg.4	Eg.3	Eg.2	Eg.1	Eg.18
To.12	0.86																	
To.11	0.81	0.75																
To.10	0.88	0.82	0.85															
To.9	0.86	0.79	0.80	0.82														
To.8	0.82	0.78	0.79	0.81	0.76													
To.19	0.85	0.83	0.81	0.81	0.81	0.84												
To.17	0.63	0.60	0.70	0.64	0.65	0.63	0.66											
To.16	0.66	0.62	0.71	0.69	0.70	0.64	0.65	0.77										
To.15	0.60	0.62	0.63	0.63	0.66	0.60	0.61	0.76	0.86									
Eg.7	0.61	0.57	0.62	0.60	0.61	0.58	0.61	0.77	0.85	0.87								
Eg.6	0.60	0.60	0.61	0.61	0.62	0.59	0.62	0.71	0.72	0.75	0.74							
Eg.5	0.63	0.65	0.68	0.66	0.63	0.63	0.66	0.79	0.75	0.76	0.72	0.78						
Eg.4	0.55	0.51	0.56	0.54	0.55	0.51	0.51	0.57	0.66	0.60	0.59	0.56	0.59					
Eg.3	0.58	0.50	0.59	0.57	0.57	0.51	0.53	0.64	0.65	0.63	0.60	0.61	0.64	0.77				
Eg.2	0.57	0.51	0.56	0.56	0.53	0.52	0.55	0.63	0.62	0.60	0.62	0.60	0.63	0.79	0.84			
Eg.1	0.58	0.51	0.56	0.58	0.58	0.53	0.56	0.65	0.66	0.66	0.63	0.60	0.63	0.76	0.86	0.85		
Eg.18	0.56	0.54	0.57	0.59	0.58	0.53	0.56	0.62	0.68	0.66	0.61	0.62	0.65	0.83	0.85	0.89	0.86	
Eg.14	0.50	0.44	0.51	0.49	0.49	0.47	0.46	0.51	0.59	0.57	0.54	0.53	0.58	0.68	0.72	0.74	0.71	0.72

S: Samples To.: Tomato Eg.: Eggplant

The similarity average was 65 % of all samples, it ranged between 89 % (Eg.2, Eg.18) and 44 % (To.12, Eg.14) (Table 3), these results agree with to previous study were the similarity ranged between 43 - 86 % using RAPD-PCR (Mouhanna and Barhoum 2014). Genetic diversity between samples was not affected the location, height or environment because the phylogenetic tree contained samples from the four regions (Lattakia, Jableh, Banias and Tartous) and from various environment (greenhouses, open fields up to 1200 m) (Fig. 4). The correlation between hosts (Tomato , Eggplant) and studied samples was weak and insignificant (r = 0.04, p-value >0.05), this refers that there is no effect of hosts on variation.

Many studies confirmed the ability of ISSR marker to detect the genetic diversity (Moreno, *et al*.,1998), and been often more efficient than RAPD (Sharma, *et al*., 2008). This study showed that the phylogenetic tree was divided into two clusters using ISSR markers for both tomato and eggplant. The percent disagreement values were 0.64, 0.6 and 0.57, and the variation range was 10.9 %, 11.7 % and 14.3 % for tomato, eggplant and both of them respectively. Study of Mouhanna and Barhoum (2014), using the same samples under the same laboratory conditions (quantity and quality of the DNA, PCR etc.) showed that the average of similarity for all samples were 0.58. The highest similarity was 0.86 between To.19 and Eg.18 which collected from open fields (<400 m), whereas the lowest similarity was 0.43 between (To.19, Eg.6) and (Eg.14, Eg.5). The percent disagreement values in our study were 0.50, 0.47 and 0.42, and the variation range was 24.9 %, 18.59 % and 20.89 % for tomato, eggplant and both of them respectively. Although the phylogenetic tree showed a differentiation in the samples distribution whether using ISSR or RAPD markers, however the similarity percentage was so close between ISSR and RAPD markers, There was no effect of plant hosts (tomato, eggplant) on samples where the correlation (r) was 0.04 and 0.09 using ISSR and RAPD respectively. This result is inconsistent with Sharma *et al*., (2008) who indicated an effect of plant hosts on the genetic diversity of whitefly populations after breeding for 12 generations on a specific host. On the other hand, some of the ISSR fragments were distinguishable, and could probably help to identify biotypes that could be associated with a specific or several plant hosts. Perring *et al*., (1993) showed that the range of similarity percentage was (0.8 - 1) between the populations of same biotype, and Dong *et al*., (2008) indicated a range of similarity percentage between B and Q biotypes of 0.45 - 0.79 using ISSR marker. The polymorphism in this study was 75 % using ISSR marker, and 82.31 % using RAPD (Mouhanna and Barhoum, 2014), that reinforce the efficiency of the ISSR and RAPD markers in detecting genetic diversity of whitefly populations. However, the correlation coefficient between these two markers (Mantel Test) was weak (r = 0.2).

Perspectives

Therefore, we need to include a larger number of samples and using other genetic diversity indicators such as Shannon's index, which is characterized by its insensitivity to data bias for the ISSR and RAPD markers. It would be useful to apply other specific markers to determine the biotypes of this insect such as Internal transcribed spacers (ITS1) and mitochondria cytochrome c oxidase I (mtCO1) (Aline, 2008; Dinsdale, 2010) and sequence in specific regions of their genomes.

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