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Population Genetic Structure of *Hishimonus phycitis* (Hem.: Cicadellidae), Vector of Lime Witches' Broom Phytoplasma

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ABSTRACT

Witches' broom disease of lime caused by '*Candidatus* Phytoplasma *aurantifolia*' is considered as one of the most destructive disease of Mexican lime in southern Iran, Oman, and the United Arab Emirates. The causative phytoplasma is vectored by a leafhopper, *Hishimonus phycitis* (Distant, 1908). Six ISSR markers and mitochondorial Cytochrome *c* Oxidase I (COI) gene were used to unveil genetic variation of the leafhopper populations from thirteen different regions of Iran. Analysis of ISSR markers revealed that Forg (Fars) and Qale'e Qazi (Hormozgan) significantly diverged from the other populations. However, the COI sequences were highly conserved among all populations and resided all the populations in a single clade. Mantel test exhibited no correlation between genetic and geographical distances. Our results demonstrated genetic differentiations among the *H. phycitis* populations, which might have been induced by ecological or geographical isolation and may affect the vectoring capability of this insect.

Keywords: COI, Genetic differentiations, I SSR markers, Mitochondrial gene based marker, Vectoring capability.

INTRODUCTION

Witches' Broom Disease of Lime (WBDL), caused by "*Candidatus* Phytoplasma *aurantifolia*", has been considered as a lethal disease of Mexican lime, which is an economically important crop in Iran. The first report of WBDL dates back to the 1975 from Oman, which indicted a substantial damage to the Mexican lime orchards (Bové *et al*., 1988). Subsequently, it was observed in the United Arab Emirate in 1989 (Garnier *et al*., 1991) and, later, in Iran in 1997 (Bové *et al*., 2000). During 19 years, the disease was spread throughout the four main lime-growing provinces of southern Iran (i.e. Sistan-Baluchestan, Hormozgan, Kerman, and Fars). The outbreak results in devastating approximately 30% of the Mexican lime trees in southern Iran (Mardi *et al*., 2011). A potential candidate vector, *Hishimonus phycitis* (Distant) (Hem.: Cicadellidae) was established as the actual vector for WBDL phytoplasma on lime trees in south of Iran (Bagheri *et al*., 2009). Control of Phytoplasma diseases is difficult due to their vectors, which are of small size and highly mobile (Saracco *et al*., 2007). An integrated pest management approach that incorporated control of the leafhopper vector, monitoring of the insect, early detection and eradication of abnormal plant material should be carried out to achieve long-lasting and sustainable

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control of this insect vector (Marzachi *et al*., 1999; Naseri *et al.,* 2009).

A sustainable pest management program for WBDL in Iran can rely on accurate information from the vector, *H. phycitis* populations. Determining genetic structure of *H. phycitis* populations in various climate zones is a fundamental piece of information to establish a vector base management strategy for WBDL (Bové *et al*., 2000). Knowledge of population genetic structure of *H. phycitis* may pave the way for understanding different aspects of biology and ecology of this insect vector. In addition, it may shed light on gene flow among the populations of this insect, which may be used in vector-based management strategies of WBDL.

The DNA-based molecular markers have been widely used as a tool to assess genetic diversity in number of insect species (Behura, 2006). Shabani *et al*. (2013) previously analyzed the genetic structure of *H. phycitis* population using mitochondrial Cytochrome *c* Oxidase I (COI) and nine microsatellite DNA marker. They only tested seven populations (one from Oman, six from Iran) with not enough variation. The former study could not separate various WBDL-vector populations which may stem from their inability in discrimination of low genetic differences. It has been documented that ISSR technique has resolved some challenging issues in which the other molecular markers like RAPD, SSR, IRAP and REMAP failed (Grover and Sharma, 2016). As in genetic studies, especially about vector of serious plant diseases, even narrow genetic differences should be considered.

In this study, we exploited six ISSR markers to uncover genetic differences among thirteen WBDL-vector populations distributed in four provinces of Iran. A mitochondrial gene based marker (COI) was also tried to unveil any possible highly diverged population and to compare the ISSR with a gene based marker.

MATERIALS AND METHODS

Insect Collection and DNA Isolation

Adult *H. phycitis* leafhoppers were collected from lime orchards using a D-Vac aspirator (Echo-ES210; Japan) from 13 sites in four southern provinces of Iran during May and June, 2015 (Table 1). Specimens were preserved in acetone (Fukatsu, 1999) and stored at -20 ºC for further studies.

Total DNA was extracted from the individual leafhoppers of each population using a CetylTrimethyl-Ammonium-Bromide (CTAB) method in accordance with an adapted protocol from Reineke *et al*. (1998). The quality of the extracted DNA was verified on a 1% agarose gel and amount of the total genomic DNA was quantified using a NanoDrop. The isolated DNA was stored at -20 ºC.

ISSR Amplification and Gel Electrophoresis

The extracted genomic DNA was used as template in the PCR reactions for ISSR analyses. To study *H. phycitis* diversity, total numbers of eight ISSR primers were used (de León and Jones, 2004). Six ISSR primers which produced reproducible and clear bands across all populations were selected for diversity analysis (Table 2). The ISSR reactions were performed in 10 µL [25 ng of template DNA, 0.2 mM dNTPs, 0.5 µmol primer and 1.0 µL of 10X PCR buffer] (Cinnagen, Iran). The amplifications were performed on a Universal Gradient Peqlab 96 wells thermal cycler with reaction conditions programed as initial pre-denaturation at 95°C for 2 minutes followed by 40 cycles of denaturation at 94°C for 1 minute, specific annealing temperature for 1 minute, and extension at 72°C for 2 minutes with a final extension at 72°C for 10 minutes. After amplification, the DNA fragments were separated by electrophoresis onto 2% Agarose gel submersed in 1X TAE

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Table 1. Localities of sample collection sites for *Hishimonus phycitis* populations from *Citrus aurantifolia*, codes and coordinates of collection sites, and the corresponding infection rates for the disease (WBDL).

Locality	Code	Number/Sex	Latitude	Longitude	Infection rates of localities for WBDL[a]
Hormozgan/Roudan	Hr	2♂, 8♀	N27º44'50"	E57 º 13' 75"	High
Hormozgan/Minab	Hm	6♂, 11♀	N27 º 32'18"	E57 º 10' 05"	High
Hormozgan/Hashtbandi	Hh	8♂, 9♀	N27 º 05'01"	E57 º 23' 75"	High
Hormozgan/Qale'e Qazi	Hq	4♂, 6♀	N24 º 45'00"	E56 º 54' 58"	Low
Hormozgan/Sirmand	Hs	2♂, 3♀	N27 º 98'20"	E56 º 12' 36"	Medium
Hormozgan/Tashkooyeh	Ht	5♂, 3♀	N28 º 14'66"	E55 º 44' 83"	High
Kerman/Kahnouj	Kk	7♂, 4♀	N27 º 97'75"	E57 º 71' 15"	High
Kerman/Jiroft	Kj	4♂, 13♀	N28 º 67'68"	E57 º 68' 52"	High
Kerman/Ali Abad	Kaa	5♂, 5♀	N28 º 58'93"	E57 º 84' 31"	Medium
Kerman/Manoojan	Km	6♂, 4♀	N27 º 51'96"	E57 º 55' 30"	Medium
Kerman/Jahad Abad	Kja	3♂, 4♀	N28 º 54'71"	E57 º 86' 91"	Medium
Sistan-Baluchestan/Nikshahr	Sn	2♂, 2♀	N26 º 14'13"	E60 º 44' 27"	Low
Fars/Forg	Ff	2♂, 1♀	N28º 16'23"	E55 º 14' 20"	Low

[a] The infection rates of localities for WBDL means also the density of the vector.

Table 2. ISSR primers information used in genetic structure of *Hishimonus phycitis.*

Primer code	Sequences (5′ – 3′)	TBN[a]	PBN[b]	P%[c]	PIC[d]	MI[e]
P6	$HVH(TG)_7T$	31	31	100	0.419	12.989
P9	$CCAG(GT)_7$	33	33	100	0.387	12.771
P10	$G(TG)_4(AG)_4A$	34	34	100	0.357	12.138
P13	$A(CA)7(TA)_2T$	29	28	96.5	0.349	9.772
P15	$T(GT)7(AT)_2$	33	33	100	0.398	13.134
P16	$KKVRVRV(TG)_6$	41	41	100	0.388	15.908
Total		201	200	99.5	0.369	73.800

[a] Total Band Number; [b] Polymorphic Band Number; [c] Polymorphic Percent; [d] Polymorphic Information Content, and [e] Marker Index ; (MI). K: G/C ; V: G/C/A ; R: G/A, H: A/T/C.

buffer for 2 hours. The DNA stained using FluoroDye (DL5000, DNA Fluorescent, Loading Dye, Green, 6x). DNA fragments were visualized and documented using, Uvitec Geldoc system. The DM2300 DNA ladder (100 bp+3k) was used as a standard molecular weight marker.

Scoring the Band and Data Analysis

ISSR products were translated to numerical data as either 1 (present) or 0 (absent) band in a spread sheet. A pair-wise similarity matrix was constructed using the Jaccard, Simple Matching (SM) and Dice similarity. Polymorphism Information Content (PIC) was calculated using the following equation (Roldan-Ruiz *et al.* (2000):

$$PICi = 2fi\ (1-fi) \quad (1)$$

Where, *fi* is the frequency of the amplified allele (present band), and (1-*fi*) is the frequency of the null allele (absent band). Marker Indices (MI) were calculated as the product of PIC and the number of polymorphic bands per ISSR primer, as suggested by Powell *et al*. (1996). Principal Coordinate Analysis (PCoA) was performed based on ISSR data using R package.

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Amplification of mtDNA

To extend our observation, we also examined the variation of the mitochondrial gene Cytochrome *c* Oxidase subunit I (COI) of Forg (three samples) and Qale'e Qazi (four samples) populations as we found these populations diverged from others in ISSR assays. The fragment *ca.* 670 bp was amplified by PCR using primer LCO1490 and HCO2198 (Folmer *et al.*, 1994). The PCR was performed in 25 µL solution containing 12.5 µL Master Mix, 1 µL of each primer (10 pmol μL^{-1}), 1 µL of extracted DNA and 9.5 µL double-distilled water. The thermocycling program consisted of an initial denaturation step at 95°C for 3 minutes, followed by 5 cycles of 1 minute at 94ºC, 1 minute at 45ºC, 1 minute at 72ºC, and then 35 cycles of 1 minute at 94ºC, 1 minute at 51ºC, 1 minute at 72ºC, with a final extension step at 72ºC for 5 minutes. All PCR products were directly sequenced with both primers by Macrogen Sequencing Service (South Korea). Sequences used in phylogenetic analyses were checked and aligned using software: DNAstar and ClustalX. The COI sequences of *H. phycitis* were deposited in GenBank under accession number KY654338-44. Other sequences used in comprehensive phylogenetic analyses were downloaded from GenBank. *Orosius albicinctus* Distant, 1918 COI sequence was used as out group in all analyses.

We used ARLEQUIN version 3.5.1.2 (Excoffier and Lischer, 2010) to estimate Analysis of Molecular Variance (AMOVA) (Excoffier *et al.*, 1992). Significance associated with the fixation index was evaluated through a non-parametric permutation procedure (10,000 permutations) using the same program. Nei's standard genetic distance was calculated using the same program. Statistical calculations and graphics for F_{ts} (Weir and Cockerham, 1984) were calculated using the same program.

The parsimony analysis on the entire data was performed using PAUP V. 4.0b10 software (Altivec). A maximum parsimony heuristic search comprising 10^4 random-sequence were applied with Tree-Bisection-Reconnection (TBR) branch swapping, and MulTrees switched off. Parsimony settings were AccTran and collapse of zero length branches. Additional branch swapping on the most parsimonious trees was performed with MulTrees in effect, and the remaining setting as before and the remaining settings were performed like before. The output file of the phylogenetic program was visualized and re-drawn using Dendroscope V.3.2.8 and CorelDRAW V.X7, respectively.

The model of base substitution was opted using MrModeltest2 (Neylander, 2004). According to the Akaike criterion, a general time reversible model, which includes among-site rate heterogeneity and estimates of invariant sites (GTR+G+I), was used in phylogenetic analyses. Phylogenetic tree was drawn using MrBayes V.3.1.2 (Ronquist and Huelsenbeck, 2003). The remaining samples, after discarding burn-in (25% of the samples) samples and evaluating convergence, were retained for further analysis. To establish the equilibrium distribution and to estimate the Bayesian Posterior Probabilities (BPPs) of clades (Larget and Simon, 1999) using the 50% majority rule, we used the Markov Chain Monte Carlo (MCMC) method within a Bayesian framework, running for 10 million generations. The BPP values higher than 0.50 were set on appropriate clades. We applied Dendroscope V.3.2.8 and CorelDRAW V.X7 to visualize and re-draw the output phylogenies.

We calculated matrices of genetic distance data $[F_{ST}/(1-F_{ST})]$ and logarithms of geographical distance (ln Km) between all the sampling sites to find if isolation-by-distance reflected effects on genetic structure of the populations. Mantel test, with 10,000 randomization, was used to test the degree of correlation of these matrices (Mantel, 1967). This analysis was conducted by the web-based program Isolation by

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Distance web Service, IBD version 3.23 (Jensen *et al*., 2005).

RESULTS

Analysis of ISSR data exhibits a reasonable polymorphic rate as six out of eight showed polymorphic bands. In total, 201 bands were produced in which 200 were polymorphic (99.5%) (Table 2). Jaccard similarity coefficient (0.974) had higher value than SM (0.958) and Dice (0.959) coefficients according to cophenetic coefficient analysis. The ISSR markers revealed 99% polymorphic bands, with a mean of 33.3 polymorphic bands per primer. Polymorphic percentage rate (P%) varied from 100 (P6, P9, P10, P15, P16) to 96.5% (in P13), so that the mean of polymorphic percent was 99.41%. P13 had the lowest marker index (MI) value (9.772) and P16 had the highest MI value (15.908) showing high efficacy of the latter primer. The lowest and highest PIC values were obtained by P13 (0.369) and P6 (0.419), respectively (Table 2). Average PIC value for the used primers was 0.383.

Phylogenetic analysis and related tree illustrated a clade for Kahnouj (Kk) and Roudan (Hr) populations with 61.5% BootStrap (BS) value. In addition, Nikshahr (Sn) and Qale'e Qazi (Hq) were placed in a similar clade with 63.6% supports value. However, other populations diverged apart and formed solely resided clades with BS values ranging from 88 to 100% (Figure 1). Principle Coordinate Analysis (PCoA) demonstrates the genetic divergence of *H. phycitis* in two-dimensional space (Figure 2). The first axis accounts for 17% of the total variation, which separates populations Forg (Ff) and Qale'e Qazi (Hq) from the remaining populations. The second (21% of

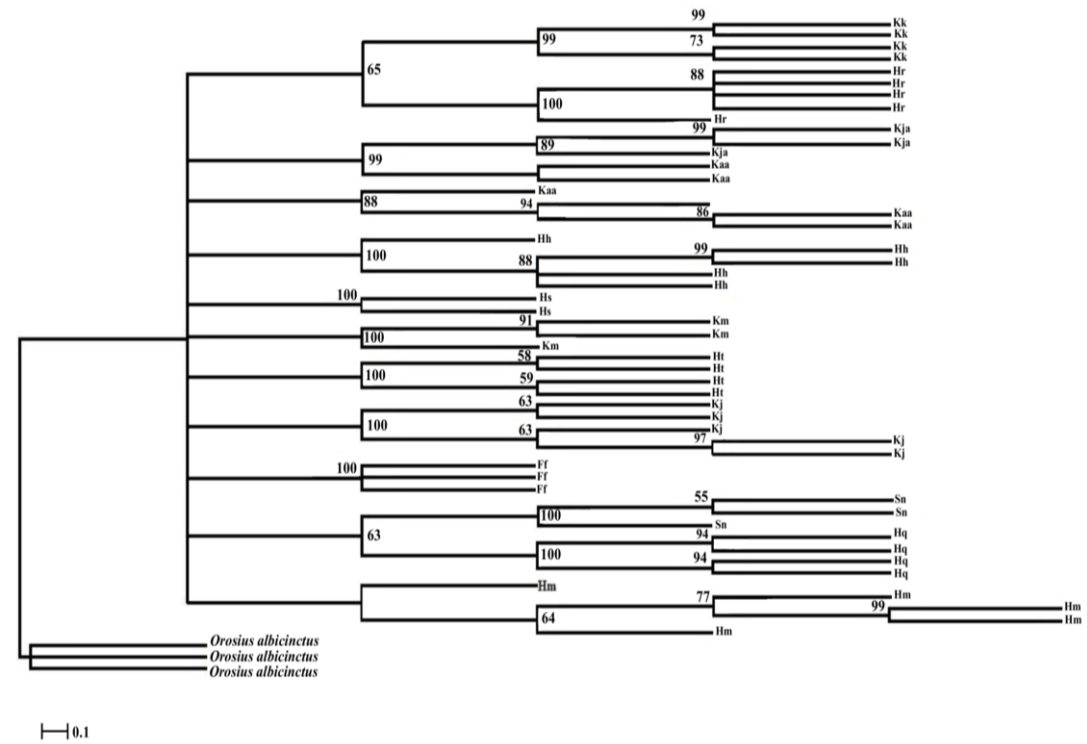

Figure 1. Dendrogram designed for thirteen populations of *Hishimonus phycitis* using ISSR markers. See Table 1 for population abbreviations.

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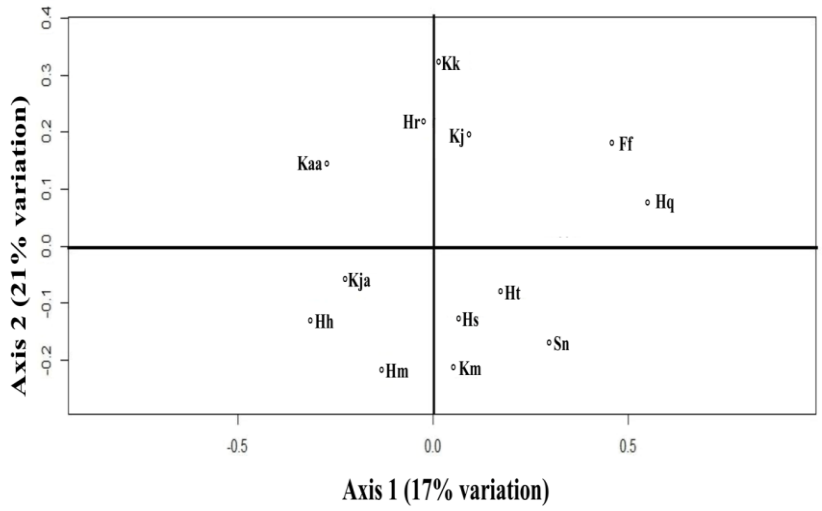

Figure 2. Principal Coordinate Analysis (PCoA) based on ISSR data from the evaluated thirteen populations of *Hishimonus phycitis* in Iran. See Table 1 for population abbreviations.

total variation) has the same result and mainly distinguishes Ff and Hq from the others (Figure 2).

Phylogenetic tree inferred from COI data showed low genetic differentiation among various populations of the leafhopper. There were no significant divergence among our sequences and those downloaded from GenBank and placed all 34 haplotypes (seven from this study and 27 deposited in GenBank) in one clade. These results indicate the existence of high gene flow among the vector populations (Figure 3).

The analysis of molecular variance of populations demonstrated a pattern with significant variation in their genetic structure among *H. phycitis* populations in which most of the observed variation resided within (88.75, P< 0.001) and some among (11.25, P< 0.001) populations (Table 3). *Fst* values inferred from ISSR marker are presented in Table 4 and Figure 4**.** Furthermore, on the basis of Nei's genetic distance, there were no significant genetic differences among other populations (Figure 5).

An *r* value extracted from the Mantel test, -12.19 (P= 0.4045, for 10,000 randomization) suggested that there was no correlation between genetic and geographical distances (Figure 6).

DISCUSSION

H. phycitis has been suggested as the actual vector of a phytoplasma causing Witches' Broom Disease of Lime (WBDL) in southern Iran (Bagheri *et al*., 2009). In the present study, we tried to address genetic variation of the *H. phycitis* populations assessed by ISSR and COI gene markers collected from thirteen geographical localities in Iran. We analyzed six ISSR primers for *H. phycitis* to investigate their genetic structure. On the basis of the ISSR results, there was no significant divergence among all populations, except Forg and Qale'e Qazi populations which diverged from the others. *Hishimonus phycitis* was first reported as a new and invasive species in Oman and the Arabian Peninsula by Bove *et al.* (1993). It has been commonly assumed that *H. phycitis* has migrated from Oman to Iran, through transporting contaminated citrus plants,

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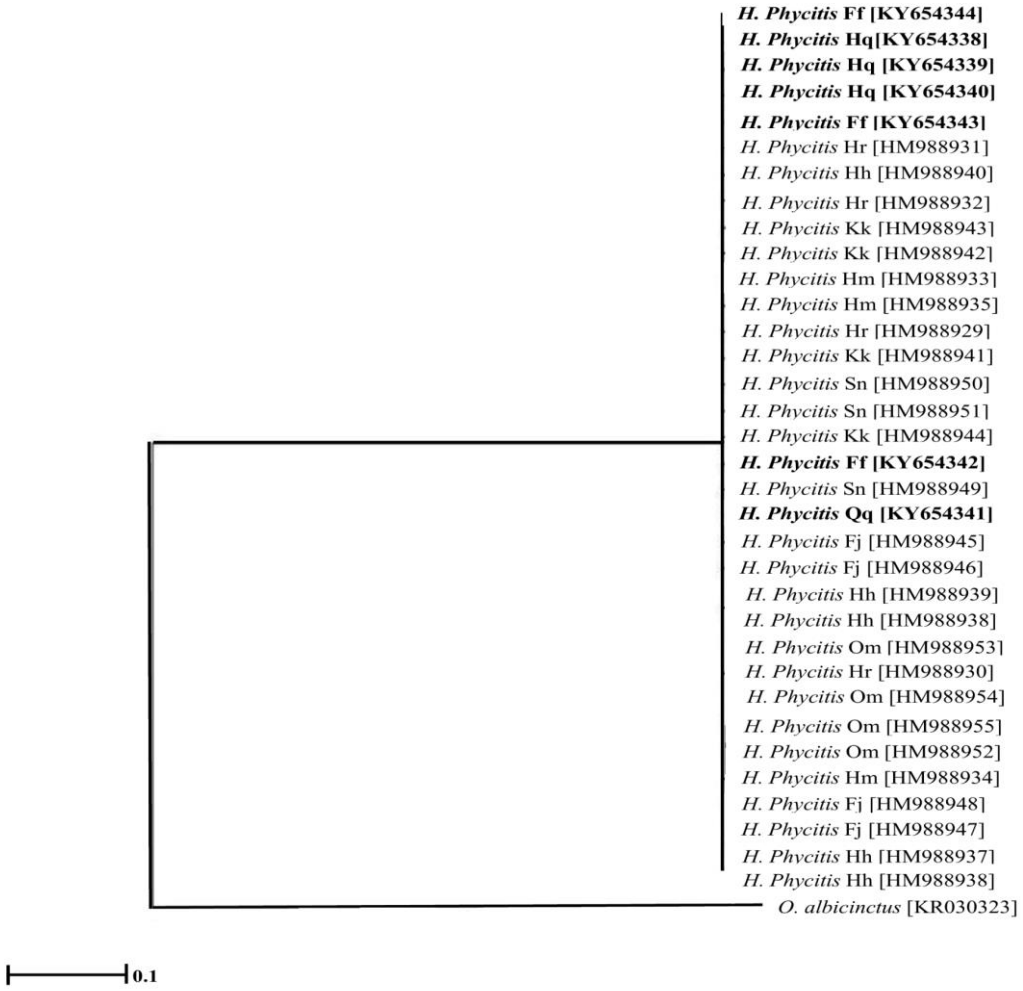

Figure 3. Phylogenetic relationship among thirteen populations of *Hishimonus phycitis* based on COI. The sequences obtained in our study are marked by bold face type. The bar indicates the number of nucleotides substitution per site. See Table 1 for population abbreviations.

Table 3. AMOVA results comparing genetic variation in *Hishimonus phycitis* collected from thirteen localities.

Source of deviation	*df*	Sum of squares	Variance components	Percentage of variation	Fixation indices
Among population	13	8.638	0.05630 va	11.25	
Within population	41	18.217	0.44431 vb	88.75	*Fst*= 0.11246, *P*= 0.0001±0.000
Total	54	26.835	0.50060		

probably due to agricultural product exchanges (Shabani *et al*., 2013). Furthermore, *H. phycitis* was found on eggplant and lime in India in 1973. Viraktamath *et al.* (1987) have concluded that the insect has been introduced to India earlier than that (at least 1500 years) based on the origin of eggplant. In addition, mycoplasma disease like little leaf is transmitted by *H. phycitis*. Shabani *et al.* (2013) have also

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Table 4. Population pairwise *Fst* of the thirteen geographical populations based on ISSR.

	Hm	Kk	Hr	Kja	Kaa	Hh	Hs	Km	Ht	Kj	Ff	Sn	Hq
Hm	0												
Kk	0.99099	0											
Hr	0.99099	0.99099	0										
Kja	0.99099	0.99099	0.99099	0									
Kaa	0.99099	0.99099	0.99099	0.99099	0								
Hh	0.48649	0.44144	0.34234	0.45946	0.34234	0							
Hs	0.99099	0.99099	0.99099	0.99099	0.99099	0.54054	0						
Km	0.99099	0.99099	0.99099	0.99099	0.99099	0.14441	0.99099	0					
Ht	0. 20721	0.44144	0.16216	0.14414	0.39640	0.16216	0.46847	0.49550	0				
Kj	0.99099	0.99099	0.99099	0.99099	0.99099	0.43234	0.99099	0.99099	0.16216	0			
Ff	0.02703	0.99099	0.0000	0.0000	0.04505	0.03604	0.06306	0.13514	0.02703	0.01802	0		
Sn	0.08108	0.02703	0.17117	0.15315	0.09910	0.14414	0.43243	0.43243	0.17117	0.09910	0.072207	0	
Hq	0.01802	0.17117	0.00901	0.02703	0.25225	0.00901	0.18919	0.22523	0.07207	0.0000	0.072207	0.09910	0

See table 1 for population abbreviations.

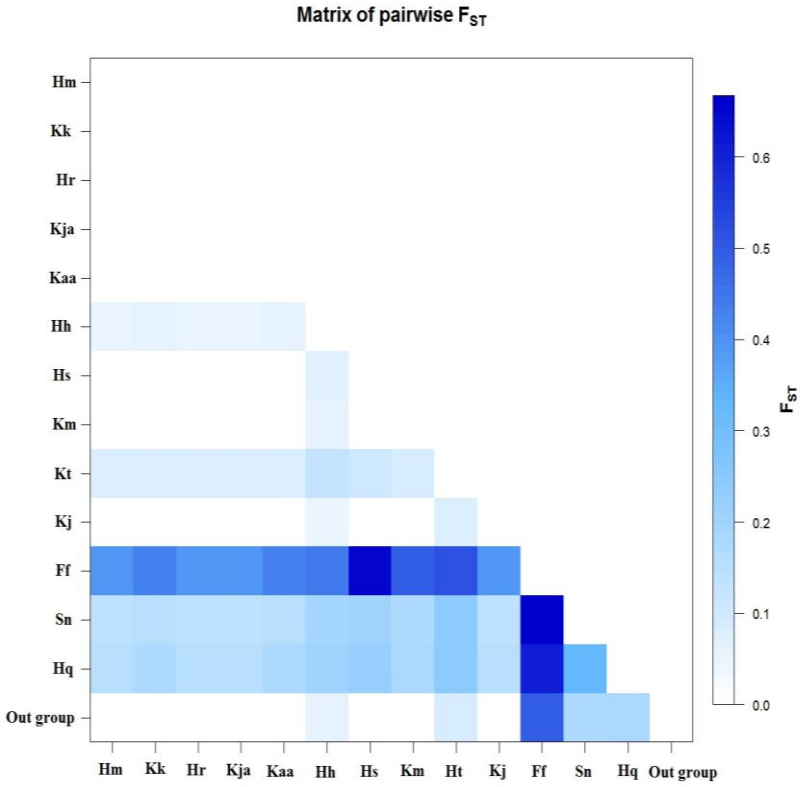

Figure 4. Population pairwise *Fst* of the thirteen geographical populations based on ISSR. See Table 1 for population abbreviations.

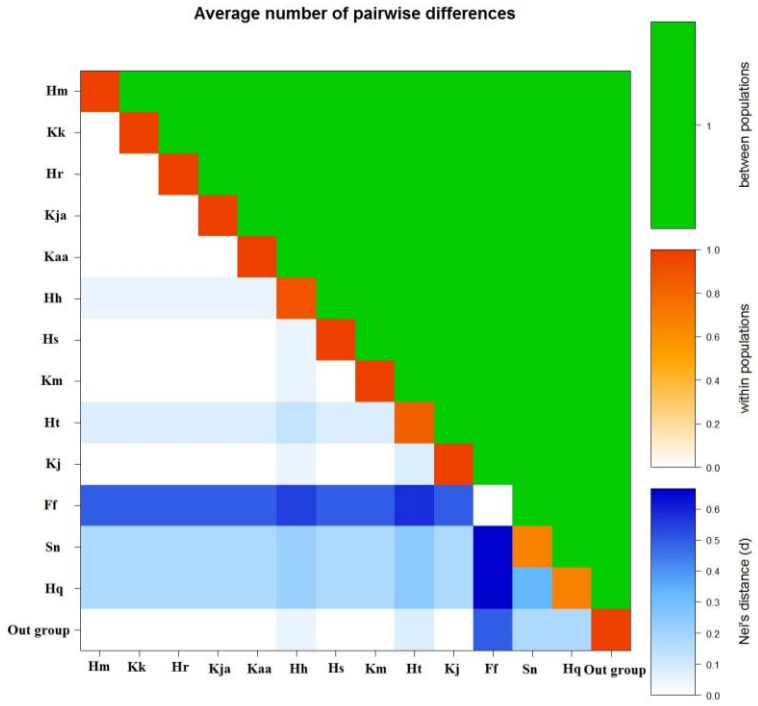

Figure 5. Average number of pairwise differences of the thirteen geographical populations of *Hishimonus phycitis* based on ISSR. See Table 1 for population abbreviations.

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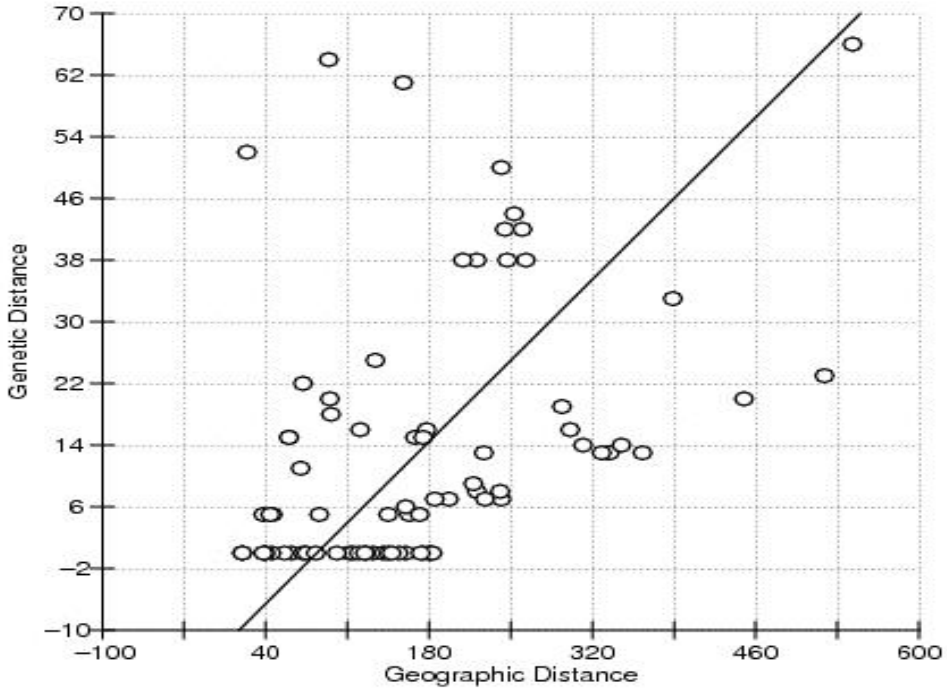

Figure 6. Plotting genetic distances derived from the ISSR analysis against geographic distances of collection sites in a partial correlation clearly showed no significant correlation between *Hishimonus phycitis* populations.

revealed that *H. phycitis* population in North Oman has become separate and distinct from those distributed in Iran. There are several factors affecting *H. phycitis* genetic structure such as extensive removal of WBDL infected host plants, application of new pesticides against *H. phycitis* and replacement of lime trees with vegetable crops (Shabani *et al.*, 2013). The AMOVA results showed that there were high levels of genetic variation within collected *H. phycitis* populations, which suggest no genetic differentiation among the populations. However, the Forg and Qale'e Qazi populations were found to be completely different and showed the highest and significant genetic distance compared with the other populations.

According to *Fst* pairwise values, the Forg (Fars) and Qale'e Qazi (Hormozgan) populations significantly differed from the other populations: low infection of the vector and consequently WBDL were found in these regions (Table 1). The existence of genetically distinct *H. phycitis* populations could have important consequences for managing this vector in the regions where lime trees are grown. For instance, *H. phycitis* populations could differ in terms of their ability to transmit *Ca.* Phytoplasma *aurantifolia* in different regions (Fars and Hormozgan) which would affects the status of *H. phycitis* as a phytoplasma vector in those regions. Furthermore, preferences for host plants, reproductive behaviour and natural enemies of this leafhopper vector might vary in different regions and, consequently, might affect its pest status and efficiency of controlling programs. More investigation is needed to try to determine the transmission rate and efficiency of the two mentioned populations. Although Mexican lime trees are endangered in all regions in which *H. phycitis* exists, due to the highly specialized and efficient transmission of the WBDL phytoplasma by this vector, approaches for managing the pest might benefit from more intensive

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monitoring of the population dynamics of *H. phycitis* in the lime-growing regions.

In a study, Shabani *et al.* (2013) demonstrated that there was a high level of gene flow between Iranian *H. phycitis* populations. This result is also supported by our findings (unpublished data) in which we identify bacterial endosymbionts hosted by this vector. We found that almost all bacterial endosymbiont were present in nearly all populations with high frequency, suggesting a high level of gene flow in Iranian populations. This finding raises a concern that a specific population with specific traits could spread in a new region and form an aggressive population which is able to spread the diseases quickly or suppress the vector activity by forming a silent population.

Symbiotic control is known as a new biological control technique for plant diseases. In this technique, symbiotic microorganisms are isolated, genetically modified, and then reintroduced to express an antipathogenic agent in the insect vector (Wangkeeree *et al*., 2012). This level of gene flow would assist us to introduce a specific gene or a transgenic population to a region and to spread these traits between populations. The technique has been used to control *Flavescence dorée* vectored by the leafhopper *Scaphoideus titanus* Ball by cross-colonizing the specific bacteria, namely, *Asaia* (Crotti *et al*., 2009). In our previous survey, *Wolbachia* was detected in all tested populations (unpublished data). This bacterium is currently being used to control dengue fever by inducing abnormal reproduction (Ruang-Areerate and Kittayapong, 2006). Taking into accounts the above facts, we can employ this bacterium to develop a new method to control this vector as well as WBDL.

Phylogenetic analysis indicated that Roudan and Kahnouj populations were placed in the same clade as Nikshahr and Qale'e Qazi (Figure 1). Sistan-Baluchestan and Hormozgan regions were the first two provinces distinguished to be infected with WBDL and the leafhopper vector in 1997-1998 (Shabani *et al*., 2013). This similarity may be due to the high level of gene exchange between the populations, which could be mediated by wind, which is the only natural phenomenon between these provinces. A northerly wind (known as 120-day wind), blowing from northeast to southwest of Sistan-Baluchestan to Hormozgan province from June to September, plays a key role in the above-mentioned displacement (Shabani *et al*., 2013). Moreover, this circular pattern of population displacement might complete by northerly winds blowing from Hormozgan to Kerman during spring and summer seasons. In a study, Zhu *et al.* (2006) indicated that flight activity and movements of small insects, having weak flying abilities, were positively affected by wind. For example, there were positive correlations between flight activity of winged aphids (green peach aphid *Myzus persicae* (Sulzer), availability of *Potato Leaf Roll Virus* (PLRV) and *Potato Virus Y* (PVY) and spring winds in the U.S northern Great Plains.

There was no significant correlation between genetic and geographical distances in *H. phycitis* populations. Bagheri *et al*. (2017) reported the lack of this correlation in Dubas bug, *Ommatissus lybicus* populations. Therefore, the other influential factors should be considered to determine genetic distance among the populations. Formerly, there were significant correlations between gene flow among the populations and human transportation of host plants, as well as other environmental factors, while geographical distances revealed no significant role (Sexton *et al*., 2013; Bagheri *et al*., 2017). In addition, as mentioned earlier, distributed populations in Iran might be spread and introduced to new regions by wind and/or infested plant residue.

In conclusion, we showed that there are variations in different populations of *H. phycitis* in Iran that may be reflected in the insect vectoring capability. These genetic variations may play a role in forming aggressive population with high pathogen transmission ability, which is a threat that

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necessitates the survey of the population variation to manage this disease. It can also be considered as a potential source to interfere with transmission of the disease and to suppress the diseases transmission rate.

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بررسی ساختار ژنتیکی زنجرک Hishimonus phycitis (Hem.: Cicadellidae)، ناقل فیتوپلاسمای بیماری جاروک لیموترش در ایران

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چکیده

بیماری جاروک لیموترش که توسط فیتوپلاسمای Candidatus Phytoplasma *aurantifolia*ایجاد می شود، یکی از مخرب‌ترین بیماری‌های درختان لیموترش در جنوب ایران، عمان و امارات متحده عربی می‌باشد که باعث نابودی سطح وسیعی از باغات لیموترش در مناطق آلوده به این بیماری شده است. فیتوپلاسمای همراه بیماری توسط زنجرک Hishimonus phycitis به درختان سالم انتقال می‌یابد .پژوهش حاضر با هدف بررسی ساختار ژنتیکی زنجرک ناقل بیماری جاروک لیموترش با استفاده از شش نشانگر ISSR و نشانگر ژنی COI انجام شد. برای این منظور 13 جمعیت جغرافیایی از چهار استان جنوبی ایران (هرمزگان، سیستان و بلوچستان، کرمان و فارس) با استفاده از دستگاه دی وک از باغات لیموترش جمع آوری و نسبت به استخراج دی‌ان‌ای از آن‌ها اقدام شد. تجزیه داده‌های ژنتیکی با استفاده از نرم افزار آرلکوین (Arlequin)انجام و درخت تباری با استفاده از نرم افزار پاپ (PAUP) ترسیم شد. نتایج به دست آمده از تجزیه داده‌های نشانگر ISSR نشان داد که دو جمعیت فورگ (استان فارس) و قلعه قاضی (هرمزگان (دارای اختلاف معنی داری نسبت به دیگر جمعیت‌های مورد مطالعه می‌باشند .نتایج به دست آمده از بررسی توالی ژن COI جمعیت‌ها، اختلاف معنی داری بین جمعیت‌های مورد مطالعه نشان نداد و همه جمعیت‌ها در یک گروه قرار گرفتند .همچنین، آزمون مانتل ارتباط معنی‌داری بین فواصل جغرافیایی و ژنتیکی در جمعیت‌های مورد مطالعه نشان نداد. نتایج ما نشان از تغییرات ژنتیکی بین جمعیت‌های این زنجرک دارد که می‌تواند به دلیل وجود جدایه‌های اکولوژیکی یا جغرافیایی باشد که ممکن است توانایی ناقل را در انتقال فیتوپلاسمای همراه بیماری تحت تاثیر قرار دهد.

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