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Genetic Structure of The Whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Tunisia, Inferred from RAPD markers

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ABSTRACT

The whitefly, *Bemisia tabaci* Gennadius, is one of the most damaging pests in agricultural systems worldwide. Knowledge of the genetic structure of this species is crucial for efficient management. In this study, we have used Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) to assess the genetic diversity and analyze the genetic structure of this insect pest. Using 120 insect samples, a total of 150 polymorphic markers were revealed. The genetic structure was analyzed, based on the molecular variance (AMOVA), genetic distances (Fst) and multidimensional scaling (MDS). AMOVA revealed that diversity between geographical domains (North vs. South) and between individuals within populations were the most determining factors in the partitioning of the total genetic diversity (50.19% and 44.85%, respectively). In addition, Fst and MDS indicated that the populations of *B. tabaci* were structured according to their geographical origin. These results strongly suggest that ecological selection pressure should be considered as a key parameter for the development of an integrated controlling strategy.

Keywords: Hemiptera, *Bemisia tabaci*, genetic diversity, RAPD-PCR, pest management.

1. Introduction

The whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), is a polyphagous pest of vegetable and ornamental crops, with a wide distribution that encompasses all tropical and subtropical regions of the world, and a reported host range including over 500 plant species [1]. *Bemisia tabaci* causes damage both directly, as a result of sap feeding, and indirectly, by vectoring virus diseases. Although populations of *B. tabaci* are, usually, morphologically indistinguishable [2]; they might vary in their characteristics, such as their ability to transmit viruses [3], rate of development [4], ability to utilize different hosts and ability to induce physiological changes in hosts [5]. These differences were used to characterize numerous biotypes [6].

Whitefly management includes cultural practices [7, 8], chemical control [9], biological control [10] and host plant resistance [11]. The integration of these four cornerstones into an integrated pest management system could significantly improve the efficiency and sustainability of control for this pest [12]. For this approach to succeed, the genetic variability and genetic structure pattern of the insect pest should be studied. For this purpose, DNA comparisons can be made between individuals or populations by means of several markers in the nuclear or mitochondrial genomes. In this context, the Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) is a relatively simple, inexpensive and rapid technique, revealing polymorphisms which are useful as genetic markers [13]. RAPD-PCR has been applied to studies on insects and to differentiate whiteflies [14, 15, 16, 17].

In Tunisia, *B. tabaci* was reported as a major pest causing severe loss to several crops, such as tomato, melon, watermelon and cucumber [16, 18]. The rapid spread of *B. tabaci* makes our knowledge of variations within *B. tabaci* populations urging for more genetic studies. The present study aims to evaluate the amount and pattern of genetic diversity of *B. tabaci* in Tunisia, on the basis of data inferred from RAPD-PCR.

2. Materials and Methods

2.1 Insect Material

Adult whiteflies were collected from host plants and fields in Tunisia, using a hand-held aspirator. A total of 120 samples were collected from 4 locations (Fig. 1) and 4 distinct crops. Insects were

identified based on the morphology of the wings ^[19]. The collected samples were preserved in 96% ethanol at -20°C for molecular analysis. Details of the studied populations are given in Table 1.

Table 1: *B. tabaci* sampling data

Sampling site	Geographical domain	Host-plant species and family	Sample size	Population label
Kébili (33°21' N, 8°51' E)	South	Lantana, <i>Lantana camara</i> (<i>Verbenaceae</i>)	20	KL
Manouba (36°48' N, 10°10' E)	North	Tomato, <i>Lycopersicon esculenta</i> (<i>Solanaceae</i>)	20	MT
		Pepper, <i>Capsicum annum</i> (<i>Solanaceae</i>)	20	MP
Korba (36°34' N, 10°11' E)	North	Potato, <i>Solanum tuberosum</i> (<i>Solanaceae</i>)	20	KP
		Tomato, <i>Lycopersicon esculenta</i> (<i>Solanaceae</i>)	20	KT
Bizerte (37°18' N, 9°52' E)	North	Tomato, <i>Lycopersicon esculenta</i> (<i>Solanaceae</i>)	20	BT



Fig 1: Map of Tunisia, showing the geographical location of the studied sites (●).

2.2. DNA extraction and RAPD-PCR.

Insect DNA was isolated according to the Cetyl Trimethyl Ammonium Bromide (CTAB) protocol described by ^[20]. RAPD-PCR reactions were performed in 25 µl volume in a mixture containing 2.5 mM MgCl₂, 1X buffer (50 mM KCl, 10 mM Tris-HCl pH 9.0), 0.1 mM of each dNTP, 0.1 µM of each primer, 50 ng of DNA and 1U of Taq DNA polymerase (Promega, USA). The amplification process was conducted in a Thermal cycler "TC-512"

(Techne, USA). For each amplification process, an initial heat denaturation of DNA at 94 °C for 3 min was performed, followed by 35 cycles consisting of 1 min at 94 °C, 1 min at 36°C and 1 min at 72°C; and incubation for 7 min at 72 °C. The amplification products were analyzed on 1.5% agarose gel in Tris-borate-EDTA buffer, stained in ethidium bromide and visualized under UV light. A 100bp ladder (Invitrogen) was used as molecular size standard. Five RAPD-PCR primers (Operon Technologies) were used; OPA-

09 (5'-GGGTAACGCC-3'), OPA-13 (5'-CAGCACCCAC-3'), OPD-12 (5'-CACCGTATCC-3'), OPD-13 (5'-GGGGTGACGA-3') and OPH-03 (5'-AGACGTCCAC-3').

2.3. RAPD data analysis.

RAPD-PCR patterns for each aphid sample were identified visually by scoring the presence or absence of all reproducible bands. The finalized fragment data from all five primers were pooled to define a single binomial phenotype for each of the 120 samples. Molecular variance (AMOVA) was determined, using ARLEQUIN software version 3.0 [21], to describe the distribution of the genetic variability between defined groups (North vs. South); between populations inside each group; and between all populations. The significance level of variance components was computed by non-parametric permutation procedures. The extent of genetic variability within a studied population was estimated by the mean genetic distance [22] calculated from the binary data for all pair-wise combinations of samples using the formula: " $D = 2n_{AB} / 2n_{AB} + n_A + n_B$ ", where n_A is the number of bands present in

individual A only, n_B the number of bands present in individual B only and n_{AB} the number of bands present in both A and B. To study the variation between *B. tabaci* populations, pairwise genetic distances (Fst) between pairs of populations were calculated and their levels of significance were tested by a permutation procedure, using ARLEQUIN version 3.0 [21]. The Euclidean distances between the six populations were then presented in a two-dimensional scaling plot using the multidimensional scaling (MDS) procedure [23] in PC SAS (SAS Institute, Cary, North Carolina, USA).

3. Results

3.1 RAPD-PCR overview.

All amplification products obtained were reproducible. For the 120 *B. tabaci* individuals, 150 DNA markers were obtained with the five primers used. Product sizes ranged from 100 to 2000 bp. All generated markers showed polymorphism between the 120 studied insect samples (Table 2).

Table 2: Total number of RAPD fragments (N), number (P) and percentage (%P) of polymorphic fragments generated, using 120 *B. tabaci* samples and 5 RAPD primers.

		N	P	%P
Primers	OPA-09	37	37	100
	OPA-13	40	40	100
	OPD-12	19	19	100
	OPD-13	19	19	100
	OPH-03	35	35	100
	Total	150	150	100

3.2 Molecular Variance (AMOVA).

AMOVA analysis obtained from the distance matrix enabled a partitioning of the overall RAPD variation within and between populations. Two groups of populations were defined according to the geographical zones; North (Manouba, Bizerte and Korba) and

South (Kebili). Results from AMOVA (Table 3) revealed a significant difference between both groups ($P < 0.01$). In addition, AMOVA showed significant genetic differences ($P < 0.01$) among individuals within populations.

Table 3: AMOVA results for *Bemisia tabaci* RAPD data.

Source of variation	% Total variation	P-value
Among groups (North vs. South)	50.19	$P < 0.01$
Among populations within groups	4.96	NS
Among individuals within populations	44.85	$P < 0.01$

NS: non-significant

3.3 Within-Population Variability.

Insect samples within each population displayed highly polymorphic RAPD patterns. The mean genetic distance between

the individuals of each population was highest for KT population (0.284), whereas it was lowest for BT population (0.181) (Table 4).

Table 4: Within-population variability of six populations of *B. tabaci* from Tunisia, estimated by the mean genetic distance [22].

Population	Mean genetic distance (D)
KP	0.219
KT	0.284
BT	0.181
KL	0.266
MP	0.253
MT	0.257
Mean	0,243

3.4. Between-Population Diversity.

Fst genetic distances between pairs of populations (Table 5) were significant; thus, it was possible to consider each population clearly differentiated. The lowest genetic distance (0.0174) was found between KP and KT, whereas the highest genetic distance (0.0914) was found between MT and KL. Multidimensional scaling (MDS) detected a clear distinction between the *B. tabaci* populations (Fig.

2). The scatter plot showed two distinct clusters: cluster I, including populations originating from the northern regions: Korba, Bizerte and Manouba; and cluster II, represented by the unique southern population of Kebili. Populations of cluster I were close genetically although they were collected on different *Solanaceae* hosts.

Table 5: Matrix of pairwise Fst between six *B. tabaci* populations based on 150 RAPD loci. Fst value are all significant (*) at the $P < 0.05$ level.

	KP	KT	BT	KL	MP	MT
KP	0.0000					
KT	0,0174*	0.0000				
BT	0,0290*	0,0304*	0.0000			
KL	0,0502*	0,0552*	0,0614*	0.0000		
MP	0,0379*	0,0364*	0,0308*	0,0763*	0.0000	
MT	0,0393*	0,0313*	0,0355*	0,0914*	0,0369*	0.0000

Values indicated in bold refer to the highest and lowest genetic distances.

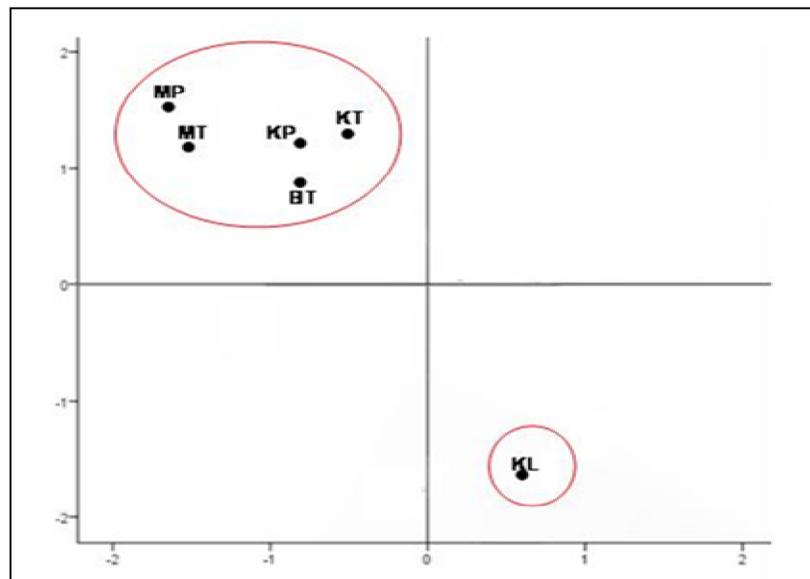


Fig 2: Multidimensional scaling scatter plot showing pattern of diversity among six *Bemisia tabaci* populations collected on 4 crops; tomato, pepper, potato and lantana, from 4 regions; Manouba, Korba, Bizerte (in the North) and Kebili (in the South), based on 150 RAPD markers. Populations are designated according to their label from Table 1.

4. Discussion

The present research focused on investigating the extent and nature of genetic variability within and among populations of *B. tabaci* in Tunisia. Six populations of *B. tabaci* were used, which were collected on 4 distinct crops (potato, tomato, pepper and lantana) from 4 sites (Korba, Bizerte, Manouba in the North and Kebili in the South). Using 5 primers, 150 RAPD markers were identified. The individuals within each population displayed a considerable genetic heterogeneity, as revealed by the mean genetic distance (0.243). This result is in agreement with most studies on *B. tabaci* populations over the world, where relatively high levels of genetic variability were detected [24, 25, 26]. Technical and biological explanations could match this high intra-population diversity. From a technical point of view, RAPD-PCR generates massive fingerprints covering a high number of loci tagged at random. Also from a biological point of view, high intra-population variability was expected due to sexual reproduction that characterizes the

studied species. Due to the anonymous nature of RAPD markers, the genetic differentiation, reported here, would not be necessarily linked to biotypes of *B. tabaci* in Tunisia, but only reflect divergence in the less functional parts of the genome that do not strongly respond to selection at physiological level [27].

Genetic relationships between *B. tabaci* populations, inferred from Fst pairwise distances and MDS, revealed that the population of Kebili collected on *Lantana camara* (*Verbenaceae*), discarded from the northern populations, which were collected on *Solanaceae* crops. This grouping pattern might be explained by an abiotic selection pressure at micro-ecological scale. Indeed, the south of Tunisia is characterized by a continental sub-Saharan dry climate, contrasting with the coastal and rainy one, characterizing the North. In Brazil, it was reported that the intensity of *B. tabaci* attack on tomato was positively correlated with mean temperature but it was not observed any significant effect of rainfall [28]. Likewise, in the Middle-East, the temperature and the relative humidity were

reported to be the most important factors relating to population changes of *B. tabaci* in the cotton fields [29]. Yet, another plausible explanation of the genetic pattern would be the contrast in host plant, between *Solanaceae* (tomato, pepper and potato) and *non-Solanaceae* (lantana). In the Hemiptera, host-based genetic structuring of populations has been frequently observed in aphid species, including *Sitobion avenae* [30], *Acyrtosiphon pisum* [31], *Aphis gossypii* [32] and *Aphis spiraeicola* [33]. Yet, in *B. tabaci*, few studies reported host-associated genetic structure, such as Abdullahi et al. [34,35], who distinguished between cassava and non-cassava-associated populations in Africa. According to De Barro [36], there would be a lack of evidence, at global scale, for the host plant playing a major role in population genetic structure in *B. tabaci*, making cassava/non cassava races, not supported by many rigorous examples. Moreover, the tendency of two populations to utilize two different hosts needs usually to be tested experimentally [36]. Therefore, we think that the genetic distance observed, in our study, between KL (Kebili, lantana) population and the northern populations collected on *Solanaceae* would be more likely related to ecological than to host adaptation.

5. Conclusions

The present study revealed that RAPD-PCR can provide a rapid and efficient tool to generate polymorphisms in *B. tabaci* and discriminate genotypes, within and between populations. The high level of intra-population variability along with the genetic distinction between the pest genotypes from the North and the South of Tunisia should be taken into account for the establishment of a database helping the adoption of appropriate management strategies. Future studies will encompass the genotypic screening of a broader temporal and geographic sample of *B. tabaci*, in comparison with known strains or biotypes.

6. Acknowledgements

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