Identification and tracking of *Bemisia tabaci* in Saudi Arabia by RAPD PCR and principal component and two-way cluster analyses

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Abstract: The whitefly (*Bemisia tabaci*) plays an important role in the transmission of plant viruses. The present study aims at evaluating the genetic diversity of *Bemisia tabaci* in Saudi Arabia by randomly amplified polymorphic DNA (RAPD). Ten populations of insects were collected on tomato and zucchini from six geographical areas in the Kingdom of Saudi Arabia, i.e. Qatif, Ha'il, Al-Hasa, Jizan, Najran, and Riyadh. RAPD-PCR employing 4 primers (OPB-20, OPA-03, OPA-10, and OPA-13) produced a total of 184 population-specific amplicons, suitable for distinguishing the ten populations of *Bemisia tabaci*. Principal component analysis (PCA) and two-way clustering were used to cluster RAPD markers (amplicons) and/or RAPD profiles (genomes). Nine components with Eigenvalues more than one were resolved by PCA with the first three components accounting for about 45% of the total variance. PCA and cluster analysis could differentiate the ten populations of *Bemisia tabaci* into two subgroups that largely corresponded to the two host plants.The insects, therefore, could be essentially clustered according to the host plant instead of the geographical region. However, the insects collected on tomato from Najran and Ha'il did not conform to this basic clustering.Within the zucchini cluster, the insects collected from Ha'il showed considerable divergence. Two-way clustering of RAPD amplicons (markers) and RAPD profiles (genomes) improved the display of groups of RAPD amplicons that cluster similarly across the profiles and showed that certain amplicons were uniquely present in certain insect populations. In conclusion, RAPD-PCR might be a useful approach for investigating the genetic variation and interpretation of the ecological distribution of *Bemisia tabaci* in the Kingdom of Saudi Arabia.

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1. Introduction

The whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), is an important viral vector in plants and has been reported in Saudi Arabia (**Ajlan *et al*., 2007**). Crop losses are often associated with the invasion and establishment of specific whitefly biotypes (**Rao *et al*., 2011**). Begomoviruses transmitted by *Bemisia tabaci* is responsible for the emergence of one of the most devastating plant diseases in the world; i.e. tomato yellow leaf curl disease (**Sax and Brown 2000; Gillespie and Roderick, 2002; Fargette *et al*., 2006; Gillespie *et al*., 2008**).

Bemisia tabaci is a complex species that lacks significant morphological characteristics to permit the recognition of behavioral and/or genetic variants. Based on mitochondrialcytochrome oxidase I gene (COI), microsatellites, ribosomal markers, and RAPD-PCR, molecular phylogeny of this complex species has identified a large number of races and genetic groups in different parts of the world and revealed the presence of new species (**Gawel and Bartlett, 1993; Frohlich *et al*., 1999; Boykin *et al*., 2007; Dinsdale *et al*. 2010**, **Delatte *et al*., 2011**).The worldwide dissemination of this disease is related to the expansion of the Middle East-Asia Minor 1 (MEAM1) B biotype or the Mediterranean (MED) Q biotype of *B. tabaci*(**Brown, 2010; De Barro *et al*., 2011**; **Kontsedalov *et al*., 2012**). Furthermore, the introduction of invasive species of *B. tabaci* into new ecosystems has provided opportunities for several begomoviruses to contaminate crops such as tomato and squash (**Péréfarres *et al*., 2012**). It has been reported previously that the distribution of biotype B into new areas in the Americas and the Caribbean is often associated with the movement of plants and the ability of some *Bemisia tabaci* populations to adapt to a broad spectrum of new hosts (**Bedford *et al*., 1994; Helmi, 2010**).However, there are few reports that explain the entry and colonization of *B. tabaci* in different areas of the world (**Carabalí *et al*., 2010**).

The present study aims at evaluating the genetic diversity of *Bemisia tabaci* in different localities in Saudi Arabia. The ultimate objective is to identify and track the various races of *B. tabaci* distributed in the kingdom of Saudi Arabia, and to recognize the

introduction/emergence of newer races of *Bemisia tabaci*. This could be very useful for the management of this invasive insect, as well as, for the interpretation of the distribution of this insect at this regional scale.A simple and cost-effective marker technology should be devised to distinguish the different genetic variants of *Bemisia tabaci*. RAPD-PCR might be advantageous as it generates comparative profiles based on randomly chosen genetic regions distributed throughout the genome (**Da Cunha Galväo, 2008**; **Sharma *et al.*, 2008**). Principal component analysis (PCA) and two-way clustering of RAPD profiles and RAPD amplicons were used to cluster genotypes according to either host plant or geographical distribution. This approach could be useful in the interpretation of the ecological distribution of *Bemisia tabaci* in Saudi Arabia.

2. Material and Methods

1. Insect populations:

The populations of *Bemisia tabaci* used in this study were collected from six regions of Saudi Arabia (Table 1).Species identification was based on morphological characteristics. Each sample was composed of 10 adult insects. All samples of *Bemisia tabaci* were kept in absolute ethanol until molecular analysis.

Table (1): Populations of *Bemisia tabaci* studied.

Host plant	Region	Population	Host plant	Region	Population
Tomato	Qatif	QT1	Squash	Al-Hasa	AS2
	Ha'il	HT1		Riyadh	RS2
	Riyadh	RT1		Najran	NS2
	Jizan	JT1		Ha'il	HS2
	Al-Hasa	AT1			
	Najran	NT1			

2. DNA and PCR:

The tubes containing insect populations were centrifuged at 10,000xg for 10 min and the absolute ethanol was discarded. The samples were washed with 500 µl of 70% ethanol and 500 µl of 30% ethanol and centrifuged at 10,000xg for 5 min. Finally, 1 ml of nuclease-free water was added to each tube to get rid of any traces of alcohol and the tubes were centrifuged again as above, and dried in a 37°C incubator for 5 min. The 10 insects in each sample were crushed together in 50 µl of tissue lysis buffer (4 M urea, 200 mM Tris, 20 mM NaCl, and 200 mM EDTA, pH 7.4), and the DNA was extracted by using the High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Roche Applied Science).

The RAPD PCR was carried out as described previously (**Gawel and Bartlett, 1993**; **Aljanabi *et al.*, 1998**). RAPD primers (Table 2) were commercially synthesized by Operon (Operon, A Qiagen Company, Qiagen GmbH, Germany). The PCR products were loaded into 2 % agarose gel in 1x TBE buffer, and the amplified DNA bands were visualized and photographed under UV light, after staining the gel with a solution of 0.5 µg/ml of ethidium bromide.

Table (2): RAPD primers used in this study.

Primer	Sequence (5' to 3')	Primer	Sequence (5' to 3')
OPB-20	GGA CCC TTA C	OPA-10	CAG CAC CCA C
OPA-03	AGT CAG CCA C	OPA-13	CAG CAC CCA C

RAPD-PCR amplification products were scored as presence (1) or absence (0) of amplicons. Band matching was based on the adjusted R_f values (AlphaEase FC Software, Alpha Innotech, CA, USA). The similarities between RAPD profiles were calculated with the band-matching Jaccard's coefficient that ranges from 0 to 1.0, where 1.0 represents 100% identity (presence and position) for all bands in the two RAPD profiles being compared. A pairwise similarity (or distance) matrix was developed and cluster analysis was performed using the Unweighted Pair Group Method with Arithmetic averages (UPGMA) method.

Principal component analysis (PCA), a mathematical procedure that uses orthogonal linear transformation, was used to recognize patterns in the RAPD-generated markers and to highlight the relationships between the profiles (genomes) examined. PCA and two-way clustering, i.e., clustering of RAPD profiles and RAPD amplicons were performed by using the the MVSP 3.2 Software (Multi Variate Statistical Package, Kovach Computing Services, Wales, United Kingdom) and 2-D Cluster and TreeView program of Stanford University, USA (**Eisen *et al.*, 1998**).

3. Results and Discussion

The four RAPD primers (OPB-20, OPA-03, OPA-10, and OPA-13) were suitable for distinguishing the ten populations of *Bemisia tabaci*. The four primers produced a total of 218 amplicons, of which 183 amplicons were found only in single populations (population-specific amplicons), while 35 amplicons were found in several populations.Out of the 183 population-specific amplicons, 57 amplicons were produced by OPA-10 primer (Table 3). This primer produced the highest percentage of population-specific amplicons among all population-specific amplicons (57/183 or 31.14%) and amplified also the highest percentage of total amplicons (66/218 or 30.27%).

Primer OPB-20 produced the highest percentage of population-specific amplicons (48/52 or 92.3%) when compared to the total amplicons produced by that primer. Out of the 52 total amplicons produced by this primer, 48 population-specific amplicons were produced (Table 4).

Table (3): Distribution of RAPD amplicons analyzed in this study.

Primer	Population–specific amplicons	Total amplicons
OPB-20	48 (26.22)	52 (23.85)
OPA-03	44 (24.04)	58 (26.60)
OPA-10	57 (31.14)	66 (30.27)
OPA-13	34 (18.60)	42 (19.26)
	183 (100%)	218(100%)

Table (4): Distribution of population-specific amplicons analyzed in this study:

Primer	QT1	HT1	RT1	JT1	AT1	NT1	AS2	RS2	NS2	HS2	Total
OPB-20	0(0)	2(3.8)	8(15.3)	3(5.7)	8(15.3)	3(5.7)	3(5.7)	6(11.5)	4(7.7)	11(21.1)	48/52(92.3)
OPA-03	0(0)	7(12.0)	2(3.4)	7(12.0)	3(5.2)	6(10.3)	4(6.9)	4(6.9)	3(5.2)	8(13.8)	44/58(75.8)
OPA-10	1(1.5)	8(12.1)	3(4.5)	6(9.0)	9(13.6)	5(7.6)	9(13.6)	6(9.0)	7(10.6)	3(4.5)	57/66(86.3)
OPA-13	2(4.8)	3(7.1)	2(4.8)	8(19.0)	4(9.5)	1(2.4)	3(7.1)	2(4.8)	4(9.5)	5(11.9)	34/42(80.9)

UPGMA dendrogram based on Jaccard's similarity coefficient showed that *Bemisia tabaci* exhibited genetic variation within and between locations in the Kingdom of Saudi Arabia.The insect population collected on zucchini from Ha'il (HZ2) had the lowest similarity to all other populations and was joined at node 9 (Figure 1). On the other hand, *Bemisia tabaci* collected on tomato from Al-Hasa (AT1), Riyadh (RT1), Jizan (JT1), and Qatif (QT1) clustered closely (joined at nodes 1, 2, and 3 in Figure 1). Interestingly, insects collected on tomato from the two most distant provinces, Qatif and Jizan, were the most similar and were joined in the dendrogram at node 1 (QT1 and JT1 in Figure 1).

The results of principal component analysis (PCA), performed on 218 RAPD-PCR amplicons showed that nine components had Eigenvalues more than one (Table 5). The first two components explained only about 32% of the RAPD variation, while the first three components explained about 45% of the total variance. Each of the remaining components accounted for about 7 to 11% of the total variance. The results of PCA analysis were in considerable agreement with the overall representation of the genomes revealed by pairwise comparisons of Jaccard's similarity coefficients. Thus, the results of cluster analysis were supported by those of principal component analysis (PCA).

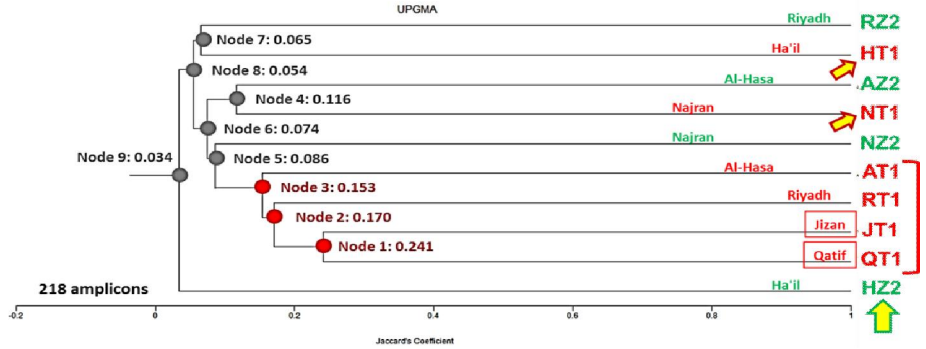

Figure (1): The dendrogram constructed from the pairwise comparisons of Jaccard's similarity coefficients and calculated based on the 218 RAPD markers. Arrows point to insects collected on tomato from Ha'il (HT1), and Najran (NT1), and insects collected on zucchini from Ha'il (HZ2).

Table (5): Principal component analysis of 10 RAPD profiles and 218 RAPD amplicons:

	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5	Axis 6	Axis 7	Axis 8	Axis 9
Eigenvalues	4.383	3.838	3.304	2.931	2.678	2.530	2.285	2.043	1.819
Percentage	16.981	14. 870	12. 799	11. 357	10. 378	9. 802	8. 853	7. 915	7. 048
Cum. %	16.981	31.851	44.650	56.006	66.383	76.184	85.037	92.952	100.00

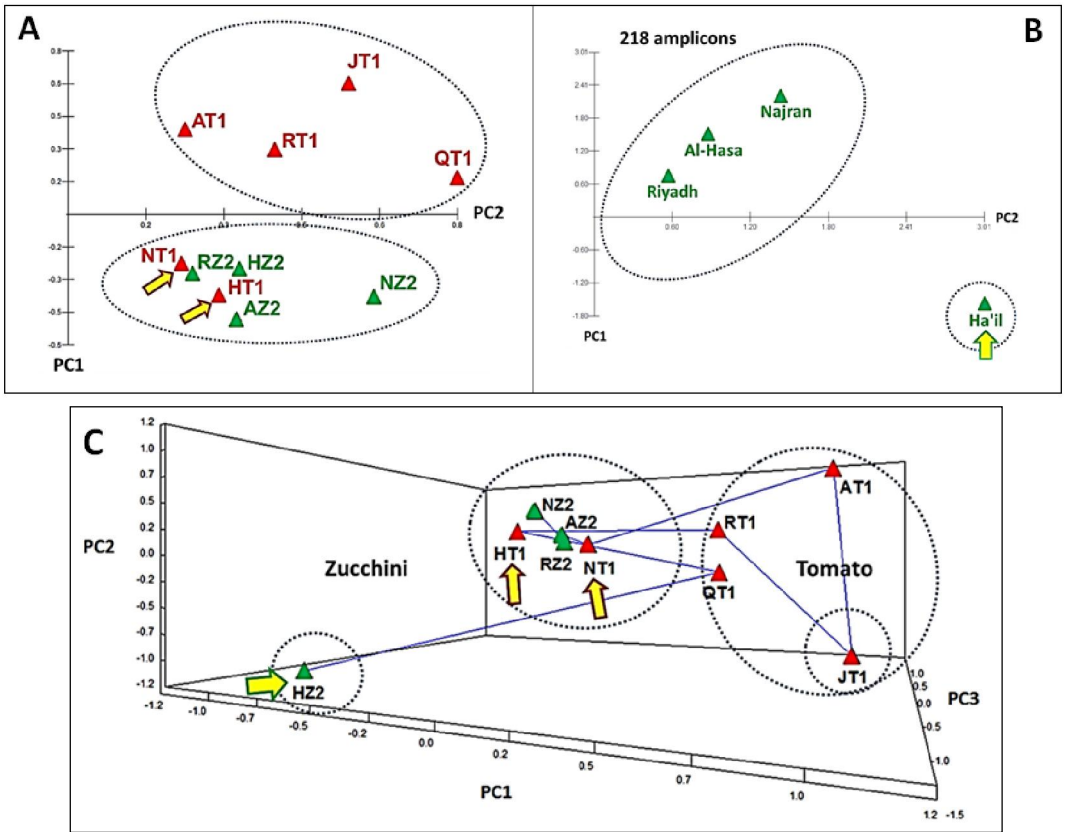

Figure (2): Results of principal component analysis (PCA) showing two-dimensional distributions (A and B) and three-dimensional distribution (C) of *Bemisia tabaci* populations. Population codes are those given in Table (1).

In the PCA plots, nearly all of the RAPD PCR types were distinct from each other (Figure 5). Two- and three-dimensional plots defined by the appropriate principal components allowed for the grouping of the 10 populations of *Bemisia tabaci* into two subgroups that largely corresponded to the two host plants. The insects collected on tomatoes from Al-Hasa, Riyadh, Jizan, and Qatif tend to cluster closely; while those collected on different hosts, but from the same location, cluster separately. The insects, therefore, could be essentially clustered according to the host plant instead of the geographical region. The insects collected on tomato from Najran and Ha'il, however, did not conform to this clustering (Figure 2a). Within the zucchini cluster, the insects collected from Ha'il showed considerable divergence (Figure 2b).The differentiation of *Bemisia tabaci* populations was also apparent by three-dimensional PCA plotting (Figure 2c).

These results suggest that the differentiation of *Bemisia tabaci* populations was mainly according to the host plant instead of the geographical region where insect populations are localized. These results were in agreement with those reported by **Lima *et al*. (2002)** and **Rabello *et al.* (2008)** who found that insect individuals were clustered with respect to host crops instead of geographical regions in Brazil. As considerable genetic variation was also found within host (between localities) in *Bemisia tabaci* individuals, this finding supports the view of invasion of new invasive members of the *Bemisia tabaci*. The genetic differentiation of *Bemisia tabaci* populations in the Kingdom of Saudi Arabia could be attributed to several factors that could have affected the allele frequencies in different populations. In addition to introduction, these may include the possible crossings between *Bemisia tabaci* individuals, and the use of crop-specific insecticides (**De Barro *et al.*, 2011**).

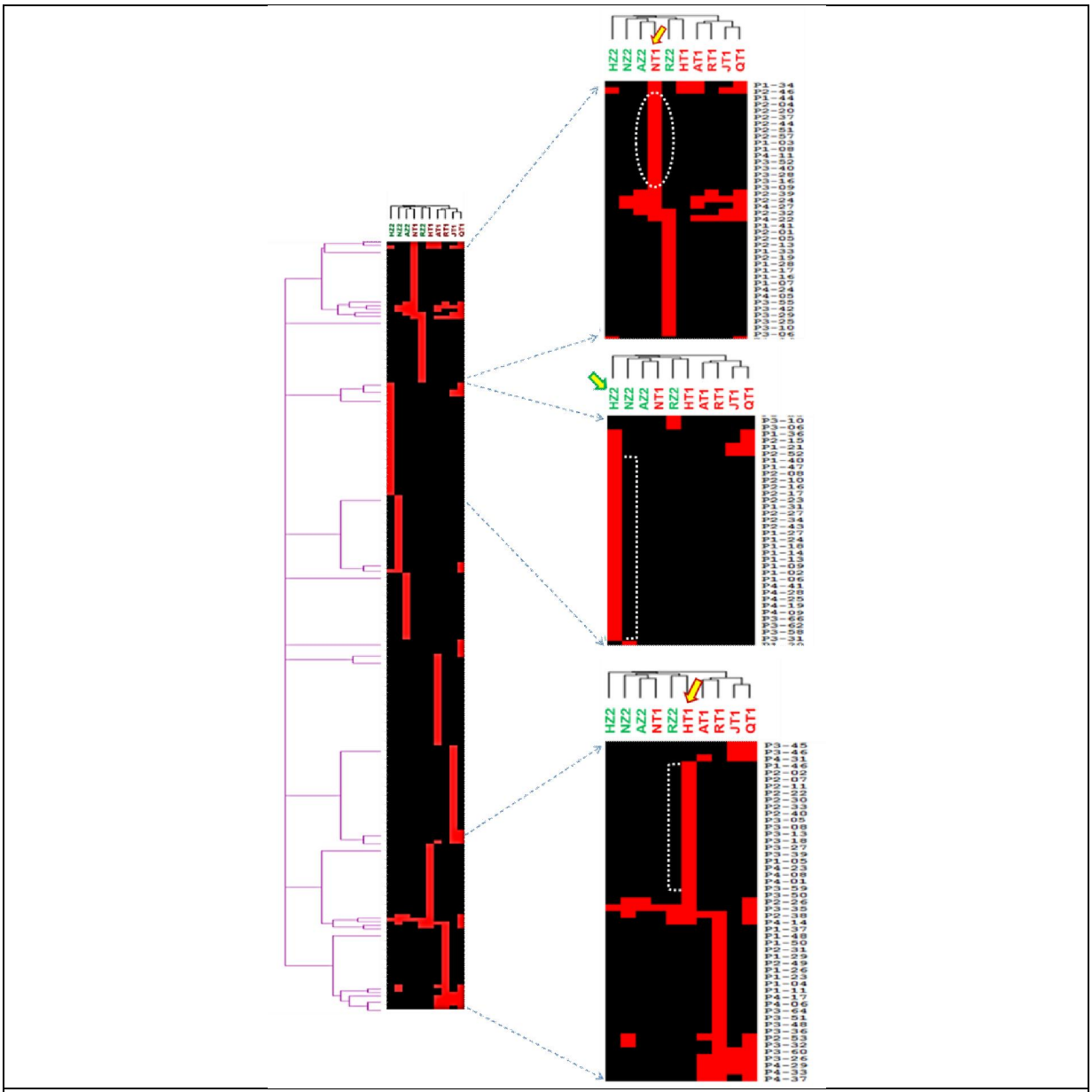

Figure (3): Two-way clustering of RAPD profiles (genomes) and RAPD amplicons (markers). Each RAPD profile is represented by a single column and each RAPD marker is represented by a single row. Each amplicon was assigned a name that begins with the letter P followed by a 2-digit number that indicates the RAPD primer (1 is OPB-20, 2 is OPA-03, 3 is OPA-10, and 4 is OPA-13), and a number that identifies the band position. Red and black colors indicate, respectively, the presence and absence of RAPD amplicons in the insect genome. Dotted oval shape and dotted left and right brackets indicate unique amplicons. The dendrograms were generated using the Cluster and TreeView program.

The two-way clustering algorithm; i.e. clustering of RAPD profiles (genomes) and RAPD amplicons (markers) was used for an easy visualization of the results as a heat map (Figure 3). In this approach, Pearson correlation coefficients are computed for all pairs of RAPD profiles (genomes) and all pairs of RAPD markers (amplicons). Hierarchical clustering based on the unweighted pair group method with arithmetic averages (UPGMA) is used to group the genomes according to the similarity in RAPD profile, and to group the amplicons according to the similarity in the pattern of amplification in the genomes studied. In the process, both RAPD profiles (genomes) and RAPD amplicons (markers) are reordered to optimize grouping, and a

pair of dendrograms is produced, one for RAPD profiles and one for RAPD markers (**Rifaat, 2011**).

Two-way clustering indicated that certain RAPD amplicons (markers) are uniquely present in certain PCR profiles (genomes). For example, the unique RAPD amplicons P1-03, P1-08, P1-44, P2-04, P2-20, P2-37, P2-44, P2-51, P2-57, P3-09, P3-16, P3-28, P3-40, P3-52, and P4-11 (dotted oval shape in Figure 3) were only present in insects collected on tomato from Najran (NT1). Likewise, unique amplicons (dotted left and right brackets in Figure 3) were present in insects collected on tomato and zucchini from Ha'il (HT1 and HZ2, respectively). These unique RAPD PCR amplicons might be useful for the identification of sequence characterized amplified regions (SCAR) markers that could be used to follow up the introduction of newly invasive members of the *Bemisia tabaci*.

In conclusion, RAPD-PCR, along with multivariate analyses and two-way clustering, might be useful for generating comparative genomic profiles that enable the evaluation of genetic diversity, the identification and tracking of *Bemisia tabaci* variants, the recognition of the introduction or emergence of newer genetic variants, and the development of cost-effective SCAR markers. Future studies should utilize mitochondrial COI bar code sequencing to carry out maternally-based phylogeographic clustering of *Bemisia tabaci* in Saudi Arabia.

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