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ARTICLE

Studying genetic diversity of whitefly *B. tabaci* Egyptian isolates in relation to some worldwide isolates

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Abstract *Bemisia tabaci* (Gennadius) (Hemiptera, Aleyrodidae) is considered to be one of the most damaging pests in agriculture, causing severe losses in crops worldwide, affecting the tropical and sub-tropical regions. Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) was used to assess the genetic diversity between different isolates collected from different regions in Egypt compared with some other worldwide isolates of this insect pest. Out of 12 primers 8 primers from Operon technology have shown to differentiate between 13 collected *B. tabaci* samples from all over Egypt and some other samples collected from different countries with two other populations representing biotypes A and B collected from the US used for biotype demarcation. Using 13 insect samples, RAPD analysis has produced a total number of 72 markers; about 68 polymorphic markers were revealed. The total number of bands obtained for each primer ranged from 4 to 14 within an average of 9 bands per primer. Of the pair wise combination among fifteen populations Ismailia population showed the highest similarity index (0.947), while US biotype A scored the lowest similarity index (0.326). Two major clusters were formed from the UPGMA dendrogram, which was constructed based on Dice similarity coefficient. RAPD-PCR screening demarcated the whitefly population based on the host species and genetic biotypes. Two major clusters have been revealed as A and B with two other minor clusters A1, A2, and B1, B2. Most of the samples collected from Egypt were clustered together in a minor cluster named A1. A1 group is divided into two sub-groups. A1a comprises the populations from Beni-Sweif in Upper Egypt, Ismailia, Kalyobia, El-Fayoum, Tanta, Kafr El-Sheikh, Alexandria, and A1b comprises Spain and Sudan. Group A1a is clustered together based on their host which belongs to the Cucurbitaceae family while Alexandria was separated individually based on its host which is cauliflower. Through the similarity matrix it could be concluded that the populations of Beni-Sweif, Ismailia, Kalyobia, El-Fayoum, Tanta, Kafr El-Sheikh had 80–90% similarity, while the Banha isolate had 30–40% similarity.

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1. Introduction

Whiteflies are insects belonging to the family *Aleyrodidae*. They occur in tropical and subtropical regions where they are pests of more than 900 hosts of horticultural and ornamental crops as well as herbaceous plants [15]. In temperate climates, they are usually pests of protected crops. About 1300 whitefly species in over 120 genera have been described [4,13] but relatively few are virus vectors.

The polyphagous sap-sucking with piercing mouthparts *Bemisia tabaci*, order Hemiptera causing many harmful effects on crop plants either directly by sucking the plant sap which causes weak plant growth and leaf chlorosis as well as wilting, or the sooty molds caused by accumulating the sugar solution produced during the feeding process of whitefly causing black spots which affect the photosynthesis and reduce the plant yield. Or the indirect damage caused by transmitting plant viruses. One-hundred and fourteen virus species are transmitted by limited genera of whiteflies. In the *genus Bemisia*, only *B. tabaci* (Genn.) is a virus vector whereas in the *Trialeurodes genus, Trialeurodes vaporariorum, Trialeurodes abutilonea* and *Trialeurodes ricini* transmit viruses. *B. tabaci* transmits 111 virus species while *T. vaporariorum* and *T. abutilonea* transmit three species each. *B. tabaci* and *T. vaporariorum* are present in the European–Mediterranean region, though the former is restricted in its distribution to the Southern parts of Europe up to the South of France. Of the whitefly transmitted virus species, 90% are begomoviruses, 6% criniviruses and the remaining 4% are in the genera *Closterovirus, Ipomovirus* or *Carlavirus* [10].

Virus transmission by *B. tabaci* is responsible for yield reduction reaching 68% in tomato as has been observed by Aboul-Ata et al. [3], five percent of viruliferous whiteflies led to 46% TYLCV infection. The same percentage of whiteflies led to 68% TYLCV infection tomato field crop in Egypt. In Pakistan Cotton leaf curl disease, from 1992 to 1995 the accumulated losses in this crop in Pakistan were calculated as exceeding $5 billion [5], where cotton covers about 60% of the country's exports, with serious effects on yield and on the nation's economy. Several agricultural practices such as monoculturing and the huge pesticide usage causing reducing agricultural enemies has developed several biotypes that differentially exhibit resistance to pesticide and virulence which was observed to happen worldwide at the same time [6]. Identifying and differentiating these biotypes is a very difficult task morphologically. So molecular markers have been widely developed to identify and compare several populations from different biotypes and different locations. RAPD markers are one of the most cheap and relatively simple and rapid techniques to be used in taxonomic purposes [17]. Also RAPD markers have been identified as an efficient tool to differentiate genetically and geographically isolated population and is mostly useful to study the genetic structure of a population because they capture polymorphisms located in introns which are non coding region [9].

2. Materials and methods

2.1. *B. tabaci* population sampling

Different *B. tabaci* isolates were collected from the northern part of Egypt from 6 governorates from squash crops from Banha, El-Fayoum, Kalyobia, and Tanta. *B. tabaci* were collected from Ismailia feeding on cucumber, from Kafr El-Sheikh feeding on cotton, and from Alexandria feeding on cauliflower, while from the Upper Egypt region were collected from the Beni-Sweif governorate also feeding on squash crop. A total of 15 samples were used for the analysis while the rest of the samples were collected from Iran, Spain, Sudan, and Morocco, and from Braunschweig in Germany were collected from the Poinsettia crop (Table 1). The insects were collected by a hand held aspirator and preserved in extraction buffer in −20 °C until DNA extraction was done.

2.2. DNA extraction

Total DNA was isolated from adult whiteflies for each sample using the high pure PCR template preparation kit (Roche, Mannheim, Germany). Extractions were carried out essentially following the manufacturer's instructions with modifications according to [1].

3–5 whitefly individuals were transferred into a sterile 1.5 ml eppendorf tube and homogenized with a sterile micro pestle in 15 µl tissue lysis buffer. After addition of another 35 µl lysis buffer and 10 µl proteinase K (20 mg/ml), the whitefly homogenates were gently mixed and incubated for 1 h at 55 °C and the extraction process was completed as described earlier by Abdullahi [1]. Aliquots of the DNA preparations were analyzed by agarose gel electrophoresis to assess the integrity and the quantity of insect genomic DNA.

2.3. RAPD-PCR

To identify and to determine the phylogenetic relationship between different isolates of whiteflies, twelve random 10-mer primers, Operon A3, A5, A8, A10, B4, B10, B11, B20, C5, C10, H16 (Table 2). 9 were used for RAPD PCR analysis resulting in a reproducible banding pattern. RAPD analyses were carried out according to [1]. Amplification reactions were carried out in 50 µl reaction mix, containing a final concentration of 1.25 mM dNTPs, 25 mM $MgCl_2$, Taq polymerase 3U/µl, 5 µl of 10× Taq polymerase buffers, 1 µl of 10 µM Primer, and DNA of 5 µl then the reaction was completed up to 50 µl. RAPD analysis was performed using 10-mer primers purchased from Operon Technologies Inc. California, USA. Amplification was performed using a thermocycler (Biorad,

Table 1 Sampling locations of whitefly population in relation to their host plants.

Species/biotype	Location	Host
Bemisia tabaci	Banha	Squash
Bemisia tabaci	Braunschweig	Poinsettia
Bemisia tabaci	Beni-Sweif	Squash
Bemisia tabaci	El-Fayoum	Squash
Bemisia tabaci	Ismailia	Cucumber
Bemisia tabaci	Kalyobia	Squash
Bemisia tabaci	Alexandria	Cauliflower
Bemisia tabaci	Tanta	Squash
Bemisia tabaci	Kafr El-Sheikh	Squash
Biotype B	US	Squash
Biotype A	US	Squash