ISOZYMES ELECTROPHORETIC VARIATIONS AMONG HOSTS AND LOCALITIES FOR POPULATIONS OF *BEMISIA TABACI* (GENN.) IN EGYPT (HEMIPTERA: ALYRODIDAE)

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ABSTRACT: Electrophoretic patterns of two isozymes (α naphthyl acetate Esterase and Malate dehydrogenase) were analyzed to determine variations among populations of *Bemisia tabaci* (Genn.) collected from nine different host plants at Qalyubiya Governorate and randomly from six Governorates in Egypt. The two analyzed isozymes successively showed polymorphic variations among the studied populations. Phylogenetic relationships among the studied populations were established according to the patterns of these two isozymes. Results concluded that *B. tabaci* species may be has different genotypes according to different localities and hosts in Egypt.

KEY WORDS: isozymes, electrophoresis, *Bemisia tabaci*, Phylogenetic relationship.

Bemisia tabaci (Genn.) has become a prominent pest in worldwide bases especially in tropical and sub-tropical agro-ecosystems (Brown, 1994, 2000). Millions of dollars have been lost as a result of direct feeding damage and plant diseases caused by whitefly-transmitted geminiviruses (Brown, 1992; Brown et al., 1991; Cohen et al., 1992; Costa & Brown, 1991; Costa et al., 1993; Yokomi et al., 1990). The existence of biotypes or host races of *B. tabaci* was proposed in the 1950s after the discovery that morphologically indistinguishable populations of *B. tabaci* exhibited measurably different biological traits with respect to host range, host-plant adaptability and plant virus transmission capabilities (Bird, 1957; Bird & Maramerosh, 1978; Costa & Russell, 1975). There has been a long-running debate as to whether *B. tabaci* is a single species or a complex of closely related taxa. Frohlich et al. (1999) concluded that *B. tabaci* was "species complex" although it was not clear from the study as to how this conclusion was reached. Since then, the term species complex has filtered into the *Bemisia* literature. The first comprehensive assessment of the species complex idea was undertaken by De Barro et al. (2005).

In the Hemiptera, host-based genetic structuring of populations has been observed in several species of aphids including *Sitobion avenae* (Sunnucks et al., 1997), *Aphis gossypii* (Vanlerberghe-Masutti & Chavigy, 1998) and *Acyrthosiphon pisum* (Simon et al., 2003) as well as in the Diaspidid scale *Aspidiotus cryptomeriae* (Miyanoshita & Tatsuki, 2001) and the Membracid leaf hopper, *Enchenopa binotata* (Rodriguez et al., 2004).

Electrophoresis techniques have been used extensively for genetic surveys of natural populations, so several authors used Electrophoretic patterns of isozymes especially esterases to determine host associated or/and geographical biotypes of *B. tabaci* (Wool et al., 1989; Costa & Brown, 1991; Burban et al., 1992& 1993; Liu et al., 1992; Brown et al., 1994; Homam, 2000; Lisha et al., 2003).

This work aims at detecting biochemical genetic variations in *B. tabaci* populations on different host plants and localities in Egypt.

MATERIALS AND METHODS

Samples Collecting: For detect variations among host plants associated populations of *B. tabaci* adults were aspirated off or/and collected in the field as pupae and brought to the laboratory, where the adults emerged from nine host plants (squash, cotton, sweet-potato, okra, egg-plant, pea, tomato, potato and watermelon) at Qalyubiya Governorate and allowed for adult emergence using insect emergence cages at the laboratory. All individual populations were maintained continuously for three successive generations on the respective host plants which were grown in pots at rearing cages. The purity of individual populations was ensured by raising new population from the parental adults of an earlier generation after these were shifted from the old screen cages to new cages. The emerging adult females from the third generation were separated and preserved immediately at -20°C in Eppendorf tubes until DNA extraction. While for detect variations among geographical associated populations of *B. tabaci* adults were aspirated off randomly from six different Governorates in Egypt (Qalyubiya, Kafr El-Sheikh, Sharqiya, Arish, Alexandria and Aswan). Samples were deep-frozen and stored at -20 °C until analysis.

Electrophoresis: Thirty adults of each population were homogenized in Eppendorf tubes containing 50 µl of extraction buffer (0.1 M Tris-Borate EDTA buffer pH 7.0 containing 10% sucrose) by aid of a handle plastic homogenizer. Tubes were centrifuged for 10 min at 10.000 rpm at 5°C. The supernatants were transferred to new eppendorf tubes and kept at deep- freeze until use for electrophoretic analysis. Vertical polyacrylamide gel 8% was used, the gels were completely covered with electrode buffer. The electrodes were connected to power supply and adjusted at 200 V for two hours. The gels were stained after electrophoresis according to its system, staining for esterases was in 100 mM Na-phosphate buffer, pH 6.0, using ±-^{2} naphthyl acetate as substrates in the ratio ±: ^{2}= 9:1, while staining for Malate dehydrogenase was in 50 mM Tris-HCl, pH 8.5, using Malic acid as a substrate with NAD, MTT and PMS. Gels were incubated at 37 °C in dark for complete staining, after that gels were fixed in a 1:5:5 mixure of acetic acid, ethanol and water for 24 hours and rinsed with distilled water two times, then photographed. All gels were scanned using Bio-Rad GelDoc2000 to calculate the pair-wise differences matrix and plot the dendrogram among different populations.

RESULTS

1. Host plant-associated *B. tabaci* populations:

1.1. α naphthyl acetate Esterases (Est):

Electrophoretic patterns of these isozymes showed highly polymorphism across the different nine host plant-associated populations Fig. (1). the maximum number of twelve enzymatic bands was detected. The least number of bands was 5 bands found in three *B. tabaci* populations (pea, tomato and okra), while the highest number of bands was 8 bands found in populations of sweet-potato and cotton. Six bands were detected in three *B. tabaci* populations (watermelon, egg-plant and squash). While population from potato showed 7 esterase bands. Bands number 5, 8, 10 and 12 were detected to be common bands for all nine populations. Both Est1 and Est4 were specific bands for four populations (sweet-

potato, cotton, okra and egg-plant). While Est2 was specific band for populations of sweet-potato and cotton only. Also Est3 was specific band for populations of potato only.

1.2. Malate dehydrogenase (Mdh):

Electrophoretic patterns of this isozyme showed less polymorphism across the different nine *B. tabaci* populations than those detected by ±-2 naphthyl acetate Esterases Fig. (2). Only four polymorphic enzymatic bands with relative migration (Rm) ranged from 0.072 to 0.553. The highest number was three bands was detected in five *B. tabaci* populations (potato, sweet-potato, cotton, tomato and egg-plant). While two bands were detected in three populations (pea, okra and squash). Only one band was detected in *B. tabaci* watermelon population. Mdh3 with "Rf" value of 0.354 was established as a common band among the nine *B. tabaci* populations. Mdh4 was detected as a specific band for egg-plant population. While Mdh2 band was detected as a negative specific band for watermelon population.

2. Geographical-associated populations of *B. tabaci* in Egypt:

2.1. α naphthyl acetate Esterases:

Electrophoretic patterns of these two isozymes showed highly polymorphism across the different six Governorates populations, Fig. (3). the maximum bands number was eleven enzymatic bands, while the least number of bands was 4 bands was found in four Governorates *B. tabaci* populations (Aswan, Qalyubiya, Kafr-El-Sheikh and El-Wadi El-Gadid), while the highest number of bands was 7 bands was found in *B. tabaci* populations collected from Qena Governorate. Fife bands were detected in Alexandria *B. tabaci* population. Est9 band was common band for the all six populations. Both of Est6 and Est10 were specific bands for Alexandria population. While Est1 and Est11 were specific bands for Qena and Kafr-El-Sheikh populations, respectively. On other hand Est 2 was found in five populations and absent in El- Wadi El-Gadid population. Also Est 5 was found in all populations except in Aswan population. So Est2 and Est5 considered as negative specific bands for El-Wadi El-Gadid and Aswan populations, respectively.

2.2. Malate dehydrogenase (Mdh):

Electrophoretic patterns of this isozyme were not polymorphic across the different six *B. tabaci* populations Fig. (4). Only three enzymatic bands with relative migration (Rm) ranged from 0.072 to 0.394. The highest number was three bands were detected in three *B. tabaci* populations (Qalyubiya, Kafr-El-Sheikh and Alexandria). While only one band with relative migration of 0.394 was detected in the other three Governorates populations. Band Mdh3 was detected to be a common band among the six populations. While bands Mdh 1 and Mdh 2 with Rm 0.072 and 0.151 were found to be specific bands for *B. tabaci* populations of Qalyubiya, Kafr-El-Sheikh and Alexandria.

3. Phylogenetic relationship among different *B. tabaci* populations:

3.1. Host-plant associated populations:

The Phylogenetic relationship among the nine host-associated populations of *B. tabaci* was generated according to the two biochemical markers (Est & Mdh). The similarity matrix is tabulated in table (1) and the Phylogenetic dendrogram is illustrated in Fig. (5). Results of this dendrogram divided the populations into two groups, the first group contained two sub-groups with similarity percentage of 71%. The first sub-group included *B. tabaci* populations of watermelon and squash with similarity percentage of 93%, while the second sub-group divided

into two classes the first included *B. tabaci* populations of pea, tomato with similarity percentage of 93%, while the second class contained potato population only. The second group contained the other four *B. tabaci* populations. This group divided into two sub-groups with similarity matrix percentage of 84%, the first sub-group contained populations of okra and egg-plant with similarity matrix of 89%, while the second sub-group contained populations of sweet-potato and cotton with similarity matrix percentage of 91%.

3.2. Geographical-associated populations:

The Phylogenetic relationship among the six geographical-associated populations of *B. tabaci* was generated according to the two biochemical markers (Est & Mdh). The similarity matrix is tabulated in table (2) and the Phylogenetic dendrogram is illustrated in Fig. (4). Results of this dendrogram divided the populations into two clusters, the first cluster contained two sub-clusters with similarity matrix percentage of 80%. The first sub-cluster included *B. tabaci* populations of Qalyubiya and Kafr El-Sheikh with similarity percentage of 96%, while the second sub-cluster included *B. tabaci* population of Alexandria only. The second cluster contained the other three *B. tabaci* populations. This cluster divided into two sub-clusters with similarity matrix percentage of 44%, the first sub-cluster contained populations of Qena and El Wadi El- Gedid with similarity matrix of 80%, while the second sub-cluster contained population of Aswan only.

DISCUSSIONS

From these results could be concluded that *B. tabaci* species may be has different genotypes according to different localities and hosts in Egypt. These results in agreement with those obtained with Homam (2000) who detected variations among populations of *B. tabaci* collected from different hosts and localities in Egypt using esterases patterns. Also, Perumal et al., 2009 found differences among *B. tabaci* populations collected from four different host plants in various locations of Tamil Nadu using molecular analysis and reported that there are at least two different biotypes of this insect pest based on these four host plants.

The utility of general isozymes electrophoretic patterns in distinguishing among different *B. tabaci* host and/or geographical associated populations was demonstrated by Wool et al. 1989; Costa & Brown 1991; Liu et al., 1992; Burban et al., 1992, 1993; Brown et al., 1994; Legg et al., 1994; Bergh et al., 1995; Brown et al., 1995; Byrne et al., 1995; Guirao et al., 1997; Homam, 2000. Also, some authors used isozymes electrophoresis to distingish among different whitefly species Guirao et al., 1997; Prabhaker et al., 1987; Idriss et al., 1997; Oliveira et al., 1997; Helmi, 2003).

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Table 1. The similarity matrix of nine host-associated populations of B. tabaci based on banding patterns of two isozymes (Est & Mdh) at Qalyubiya Governorate.

Host plant	watermelon	potato	sweet-potato	cotton	pea	Tomato	okra	egg-plant	squash
watermelon									
potato	0.82								
sweet-potat	0.67	0.76							
cotton	0.67	0.76	0.91						
pea	0.71	0.82	0.67	0.78					
poinsettia	0.8	0.89	0.74	0.84	0.93				
okra	0.67	0.67	0.84	0.84	0.67	0.75			
egg-plant	0.71	0.7	0.86	0.76	0.59	0.67	0.89		
squash	0.93	0.89	0.74	0.74	0.8	0.88	0.75	0.78	1.0

Table 2. The similarity matrix of six geographical associated populations of B. tabaci based on two isozymes (Est & Mdh) in Egypt.

	Aswan	Qalyubiya	Kafr El-Sheikh	Alexandria	Qena	El Wadi El-Gadid
Aswan	1					
Qalyubiya	0.54					
Kafr El-Sheikh	0.4	0.86				
Alexandria	0.43	0.8	0.8			
Qena	0.5	0.7	0.5	0.5		
El Wadi El-Gadid	0.44	0.7	0.5	0.5	0.8	1

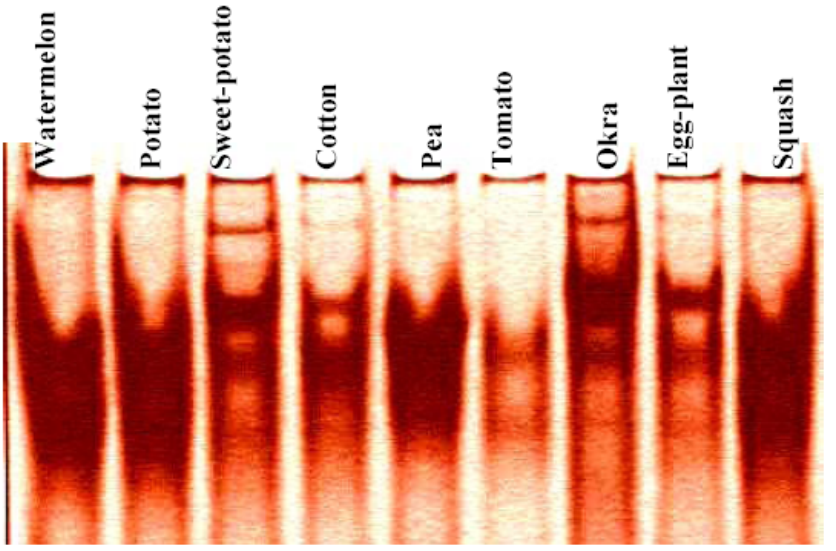

Figure 1. Polyacrylamide gel zymogram of esterase isozymes (Est) banding patterns in nine host plant-associated populations of *B. tabaci* detected with α naphthyl acetate substrates.

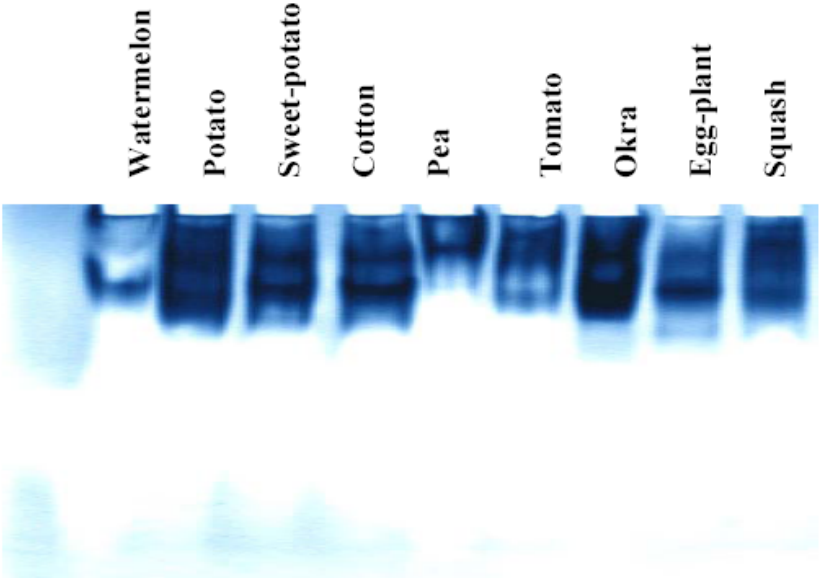

Figure 2. Polyacrylamide gel zymogram of Malate dehydrogenase (Mdh) banding patterns in nine host plant-associated populations of *B. tabaci* detected with Malic acid as a substrate.

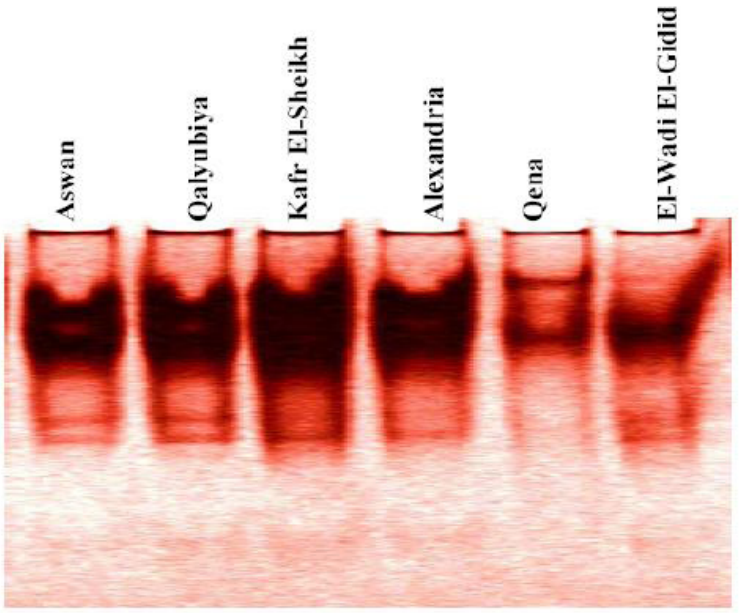

Figure 3. Poly-acrylamide gel zymogram of esterase isozymes (Est) banding patterns in six localities-associated populations of *B. tabaci* detected with α naphthyl acetate substrates.

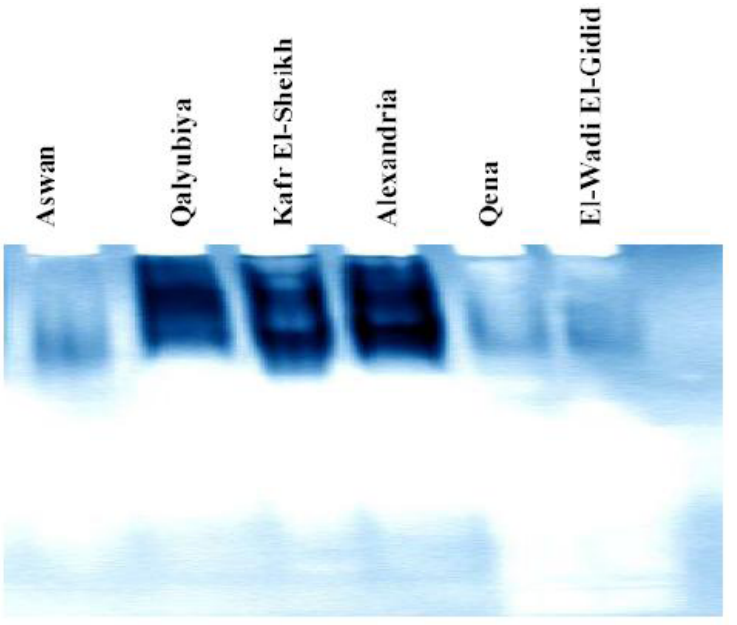

Figure 4. Polyacrylamide gel zymogram of Malate dehydrogenase (Mdh) banding patterns in six localities-associated populations of *B. tabaci* detected with Malic acid as a substrate.

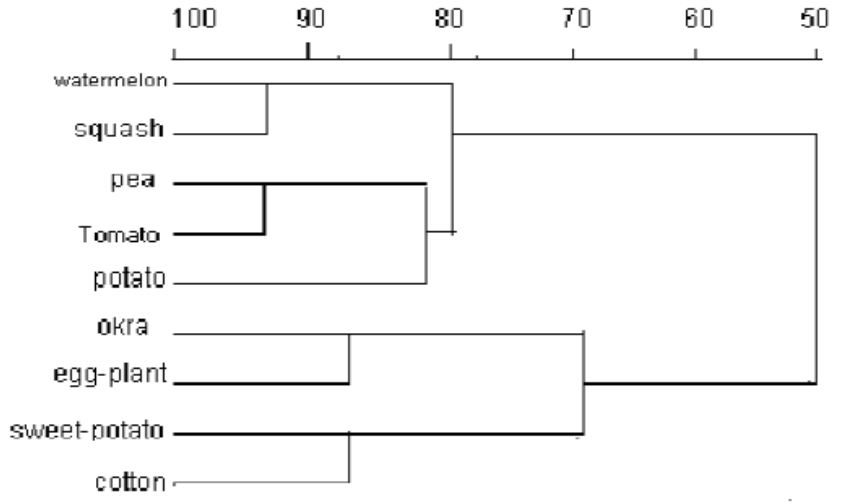

Figure 5. The similarity dendrogram of nine host-associated populations of *B. tabaci* based on two isozymes analysis (Est & Mdh).

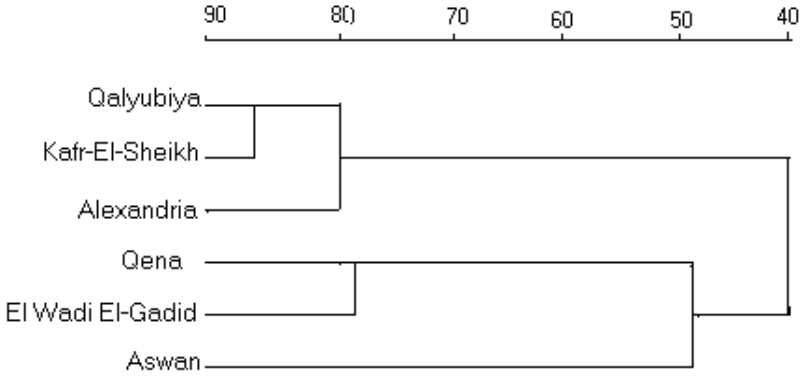

Figure 6. The similarity dendrogram of six geographical-associated populations of *B. tabaci* based on two isozymes analysis (Est & Mdh) in Egypt.