HOST PLANTS-ASSOCIATED POPULATION VARIATIONS OF *BEMISIA TABACI* (GENN.) (HEMIPTERA: STERNORRHYNCHA: ALEYRODIDAE) CHARACTERIZED WITH RANDOM DNA MARKERS.

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ABSTRACT: Whitefly, *Bemisia tabaci* is an important sucking pest of field, horticultural and ornamental plants causing feeding injuries besides spreading disease by acting as a vector of Gemini viruses. The polyphagous nature of the pest makes it as a highly complex species. The influence of six host plants utilized by the species on the population differences at molecular level was attempted using Random Amplified Polymorphic DNA (RAPD) markers. Seven RAPD primers screened produced 232 DNA fragments 223 of these fragments were polymorphic. While the other nine fragments detected as common among the six tested populations. Total number of bands obtained from each primer ranged from 23- 44 with an average of 33.14 bands per primer. Phylogenetic relationships among the studied populations using this technique clearly separated these six populations into two main clusters with similarity matrix percentage of 88% and 64%. These results indicated that *B. tabaci* may have different genotypes on adaptations to certain host plant species in Egypt.

KEY WORDS: *Bemisia tabaci*, RAPD-PCR, Molecular, Host plants, Phylogenetic relationships.

The sweet potato whitefly, *Bemisia tabaci* (Genn.), is one of the most devastating pest insects of Agriculture and horticulture in the world. It is broadly polyphagous, feeding on an estimated 900 hosts (Jones, 2003; Ma et al., 2007). Not only does *B. tabaci* cause aesthetic damage to plants, but it is also an efficient vector of a great many plant viruses (Jones, 2003; Muniz et al., 2004; Brown, 2007). An important aspect of the biology of *B. tabaci* is the high genetic variability that exists among its populations (Brown, 1994; Brown et al., 1995; Iida et al., 2009). This variability is revealed by the existence of populations that differ in their ability to feed or reproduce on particular hosts and in their virus transmission characteristics (Bedford et al., 1992 and 1994, Burban et al., 1992; Simmons et al., 2009). It was raised to the rank of a new species (Bellows et al., 1994) and was named *Bemisia argentifolii* Bellows and Perring, which produces unique symptoms of “squash silver leaf”. Recent research has tended to prove that *B. tabaci* corresponds to a species or biotypes complex in phylogenetic evolution. Its distinctive characteristics still have to be revealed by using methods different from the methods commonly used in taxonomy (Perring, 1996; Qiu et al., 2009) up to now; approximately 24 biotypes have been identified and characterized to different degrees. It was primarily divided in to B biotype, Q biotype and non B/Q biotype. The non B/Q biotypes includes more than 20 biotypes such as A, K, D, E, G, H, L, M, N. (Perring, 2001; Muniz & Nombela, 2001; Pascual & Callejas, 2004). These biotypes usually are recognized by the presence of specific phytotoxic reactions (Yokomi et al., 1990; Brown et al., 1992; Muniz et al., 2002) and

characterized by esterase markers (Costa & Brown, 1991; Costa et al., 1993; Wool et al., 1993; Brown et al., 1995; Lisha et al., 2003) and several DNA fingerprinting techniques (Guirao et al., 1997; Cervera et al., 2000; Lima et al., 2000; Mckenzie et al., 2001; Sartor et al., 2008, Sharma et al., 2008, Perumal et al., 2009; Qiu et al., 2009). Although much of the information is available on the biotype prevalence based on the locations and regions, molecular information on the genetic diversity of populations based on host plants is scarce.

This paper attempts to address our hypothesis that the population of the whitefly varies widely depending upon the host plants being utilized by the whitefly and are distinct within a narrow region or locality with poly-crop systems.

MATERIALS AND METHODS

1. Insect population samples: Whitefly pre-pupal instar were collected from the middle leaves of six host plants belonging to three families; Cucurbitaceae (Squash, *Cucurbita pepo ovifera* and Watermelon, *Citrullus vulgaris*); Solanaceae (Egg-plant, *Solanum melongena* and Tomato, *Solanum lycopersicum*) and Malvaceae (Cotton, *Gossypium barbadensa* and Okra *Hibiscus esculantus*) from Shalakan farm at Qalyubiya Governorate during 2009 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.

2. Randomly Amplified Polymorphic DNA of the Polymerase Chain Reaction (RAPD-PCR):

2.1. DNA Extraction:
DNA was extracted from adult females of each population. Thirty adult females were crushed in 1.5 ml Eppendorf tubes to extract the genomic DNA filled with 200 µl of extraction buffer (200 mM Tris-Hcl pH 8.5, 250 mM Nacl, 25 mM EDTA, 0.5% SDS). After that, 100 µl of 3 M sodium acetate pH 5.2 was added and tubes were placed at -20º C for 10 min. Tubes were then centrifuged for 5 min at 10.000 rpm and the supernatant transferred to new Eppendorf tubes. An equal volume of isopropanol was added and the precipitated DNA was pelleted by centrifugation at 13.000 rpm for 20 min. After a wash with 70% ethanol, the pellet was dried and resuspended in 100 of TE buffer (10 mM Tris-Hcl, 1 mM EDTA pH 8.0)

2.2. Polymerase Chain Reaction (PCR):
All populations were tested against seven 10-mer random primers. All primers were synthesized by (Operon biotechnologies, Inc. Germany) with the following sequences:

OPA-09: 5'GGGTAACGCC'3
OPA-12: 5'TCGGCGATAG'3
OPA-15: 5'TTCCGAACCC'3
OPA-18: 5'AGGTGACCGT'3
OPA-19: 5'CAAACGTCGG'3
OPB-03: 5'CATCCCCCTG'3
OPB-07: 5'GGTGACGCAG'3

RAPD-PCR amplifications were performed in a total volume of 25µl. Each reaction contained 2.5 µl of 10X buffer, 2.5 µl dNTPs, 2.5 µl $mgcl_2$, 3 µl primer, 1µl taq polymerase, 2 µl genomic DNA with the volume adjusted to 25 µl with distilled water. Amplifications were carried out in Techne system, England using the following program, one cycle at 95º C for 5

min, followed by 40 cycles each at 94º C for 30 seconds, 37º C for 1 min, 72º C for 2 min and final extension at 72º C for 12 min.

PCR Products were separated in 1.2 % agarose gels at 100 volts. Gels were stained with ethidium bromide and products were visualized by UV light and photographed using a Bio-Rad gel documentation system. Data analysis was obtained by Bio-Rad Quantity one software version 4.0.3.

RESULTS

1. RAPD-PCR Analysis:

To select genetically heritable markers, each individual whitefly population was sampled from the third grown generation for all the six host specific populations to identify only genetically inheritable bands/markers in the RAPD profiles. Fifteen 10-mer random primers were screened against each individual whitefly population out of them seven primers produced fragment bands. These primers are: A-09, A-12, A-15, A-18, A-19, B-03 and B-07 with the following sequences (5'GGGTAACGCC'3), (5'TCGGCGATAG'3), (5'TTCCGAACCC'3), (5'AGGTGACCGT'3), (5'CAAACGTCGG'3), (5'CATCCCCCTG'3) and (5'GGTGACGCAG'3) for the seven primers, respectively. Those amplified distinct polymorphic bands were used for studying genetic variability among different host specific populations. These seven primers amplified a total number of 218 DNA fragments 209 of these fragments were polymorphic (Figure 1). While the other nine fragments were detected as common among the six tested populations. The total number of bands generated from each primer ranged from 23 (OPA15) to 44 (OPA18) with an average on 36.33 bands per primer. The size of bands was ranged from 27 bp to 3277 bp. The total numbers of bands amplified by these seven primers for each population were 36, 30, 47, 36, 33 and 36 bands for *B. tabaci* populations on eggplant, tomato, cotton, watermelon, squash and okra, respectively. The comparative analysis of these RAPD profiles from different whitefly types resulted in identification of a number of 42 polymorphic markers holding specificity for the studies hosts populations. These genetic differences were visualized as host specific polymorphic bands and amplified from particular host specific whitefly represented host specific molecular markers for respective whitefly genotype. The highest number of these host specific markers (17 bands) was produced by OPB-03 primer, while the smallest number of these markers was one band produced by OPA-15 primer for tomato population. The total numbers of host specific markers per each whitefly genotype were 12, 7, 4, 5, 4 and 10 for populations from eggplant, tomato, cotton, watermelon, squash and okra, respectively. (Table1). The number of common bands of each two hosts-associated populations of *B. tabaci* amplified by seven random primers was ranged between 24 bands for populations on watermelon and cotton to two bands for populations on eggplant and okra (Table 2).

1.1. Primer OPA-09:

The total number of PCR products generated by this primer was 29 bands ranged from 4 to 7 bands with molecular weight (MW) ranged from 60 to 1148 bp and relative migration (Rm) ranged from 0.86 to 0.37 µm. The lowest number of bands (4 bands) was detected in Tomato *B. tabaci* population, while the highest number (7 bands) was found in okra population. No PCR fragments found in cotton *B. tabaci* population. No common bands were found among the sixpopulations.

1.2. Primer OPA-12:

The total of PCR fragments amplified by this primer were 30 fragments ranged from 2 to 7 bands with MW ranged from 27 to 923 bp and with Rm ranged from 0.86 to 0.32µm among the tested populations. The lowest number of bands (2 bands) was found in population of *B. tabaci* on squash. While the highest number (7 bands) was detected in three *B. tabaci* populations (eggplant, cotton and watermelon). Two common bands with MW of 121 and 27 bp were distinguished for the six tested populations.

1.3. Primer OPA-15:

This primer generated the lowest number of PCR fragments (23) with MW ranged from 244 to 3277 bp and with Rm ranged from 0.34 to 0.83µm. The lowest number of bands (2 bands) was found in three *B. tabaci* populations (eggplant, Tomato and cotton). While the

highest number (9 bands) was detected in watermelon *B. tabaci* population. Two common bands with MW of 1020 and 870 bp were distinguished for the six tested populations.

1.4. Primer OPA-18:
This primer generated the highest number of PCR fragments (44) bands with MW ranged from 636 to 78 bp and with Rm ranged from 0.52 to 0.91µm. The lowest number of bands (4 bands) was found in okra *B. tabaci* population. While the highest number (10 bands) was detected in eggplant *B. tabaci* population. The two bands with MW of 644 and 376 bp were considered as common bands for the six tested populations.

1.5. Primer OPA-19:
The total number of PCR fragments amplified by this primer was 30 bands with MW ranged from 1060 to 88 bp and with Rm ranged from 0.27 to 0.78µm for five populations only, while the squash *B. tabaci* population showed no bands with this primer. The lowest number of bands (4 bands) was found in eggplant population. While the highest number (7 bands) was detected in three *B. tabaci* populations (cotton, watermelon and okra). The band with MW of 88 bp was considered as a common band for the five populations.

1.6. Primer OPB-03:
The total number of PCR fragments amplified by this primer was 39 bands with MW ranged from 1877 to 97 bp and with Rm ranged from 0.26 to 0.85µm. The lowest number of bands (4 bands) was found in Tomato *B. tabaci* population. While the highest number (9 bands) was detected in cotton population. No common bands were distinguished among the six tested populations.

1.7. Primer OPB-07:
The total number of PCR fragments amplified by this primer was 37 bands with MW ranged from 627 to 64 bp and with Rm ranged from 0.48 to 0.89µm. The lowest number of bands (5 bands) was found in Tomato *B. tabaci* population. While the highest number (8 bands) was detected in squash population. Two common bands with MW of 627 and 401 bp were distinguished among the six tested populations.

2. Phylogenetic relationships among the studied *B. tabaci* populations:
Genetic similarities and genetic relatedness amongst the six host-associated populations of *B. tabaci* were based on data obtained of seven random primers as molecular markers of RAPD-PCR. These data were subjected to using SPSS computer program to support the existence of high level of genetic relatedness amongst the investigated whitefly types. This genetic relatedness was not clear amongst the plant family populations (Fig. 2). While, relatedness dendrogram was indicated two main clusters with similarity matrix percentage of 54%. The first cluster was divided into two sub-clusters; the first one included squash and watermelon with similarity matrix percentage of 88%; the second sub-cluster included okra and tomato *B. tabaci* populations with similarity matrix percentage of 74%. While the second cluster was included cotton and eggplant *B. tabaci* populations with similarity matrix percentage of 64%. From this similarity dendrogram it could be concluded that the relationship among plant families and *B. tabaci* populations was not clear except in plants belonging to family Cucurbitaceae that showed a high similarity matrix. While this similarity matrix was not so in the plants belonging to the other two families requires further biological studies. Also there was host specificity amongst under study *B. tabaci* populations based on molecular markers amplified by these seven random primers.

DISCUSSION

Random amplified polymorphic DNA polymerase chain reaction (RADP-PCR) is a relatively simple, inexpensive and rapid technique, revealing polymorphisms which are useful as genetic and taxonomic markers (Welsh & McClelland, 1990). RAPD has been applied to study of insects (Haymer, 1994) and to differentiate whiteflies, including the identification of different biotypes of *B. tabaci* (Guirao et al., 1997; De Barro & Driver, 1997; Cervera et al., 2000; Moya et al., 2001).

Sharma et al. (2008) detected genetic variability due to host plants in *B. tabaci* populations that collected from six different host plants using RAPD-PCR. They mentioned that the whitefly types holding specificity for some host plants were studied and cited that the sequence information on these RAPD-DNA markers can be used to design more efficient, specific molecular markers with specificity to different host plants.

Perumal et al. (2009) found differences among *B. tabaci* populations collected from four different host plants at the same ecosystem were found at the same point of time but collected from various locations of Tamil Nadu using RAPD-PCR. They reported that there were at least two different biotypes of this insect pest based on these four host plants. While, Frohlich et al. (1999) stated that *B. tabaci* populations and their respective host plants occur in the same geographical region at the same point of time the identified genetic diversity appears to be a rare example of the evolutionary transition leading to sympatric speciation.

In this respect, Sharma et al. (2008) found three groups of host specific populations of *B. tabaci* on six host plants grown in the same locality and in the same time based on RAPD-PCR using nine random primers. While many studies of geographic distributions of *B. tabaci* genetic groups around the globe. This level of genetic diversity had identified at least 33 different biotypes (Perring, 2001; Simon et al., 2003; Zang et al., 2006). The global phylogenies of geographic groups of *B. tabaci* suggested that *B. tabaci* was a cryptic species complex. The overall analysis suggested that there were at least 24 species making up the complex. (Boykin et al., 2007; Dinsdale et al., 2010).

This study confirms that there exists population isolations based on host plants among the whitefly, *B. tabaci* population. Therefore, this point needs further molecular analysis to understand the physiological and evolutionary relationships, which may through some lights for taxonomical perspectives and pest management decisions. Also these results need further biological studies such as the ability to transmit plant viruses (Brown, 2007), the rate of female fecundity (Iida et al., 2009; Jing et al., 2010) and the ability to accelerate pesticides resistance (Horowitz et al., 2005).

CONCLUSION

From the above results it could be concluded that genetic variability assessment in *B. tabaci* populations originating from different host plants at Qalyubiya Governorate, Egypt indicates that the population is diversified based on the host species. Clustering pattern observed in the dendrogram showed that at least two distinct biotypes exist among the populations collected within the narrow region of Egypt. These differences may be influencing the virus vectoring capabilities of the whitefly population and also their susceptibility to insecticides, which needs further studies.

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Table 1. RPD molecular markers (bp) associated with host specificity in *B. tabaci*.

primer	Eggplant	Tomato	Cotton	Watermelon	Squash	Okra
A-09	799	411		387		1148
	464			343		628
	188			157		
A-12	369	55				157
A-15		414				
A-18	548	532				
	92					
A-19	657		780			356
B-03	459	483	883	415	798	687
	216		483	118	508	562
	124				137	437
	97					278
						145
B-07	321	359	99		64	147
		205				
Total	12	7	4	5	4	10

Table 2. The number of common bands between each two hosts-associated populations of *B. tabaci* amplified by seven random primers.

	Eggplant	Tomato	Cotton	Watermelon	Squash
Eggplant					
Tomato	8				
Cotton	12	5			
Watermelon	2	9	12		
Squash	5	10	13	24	
Okra	8	11	15	12	15

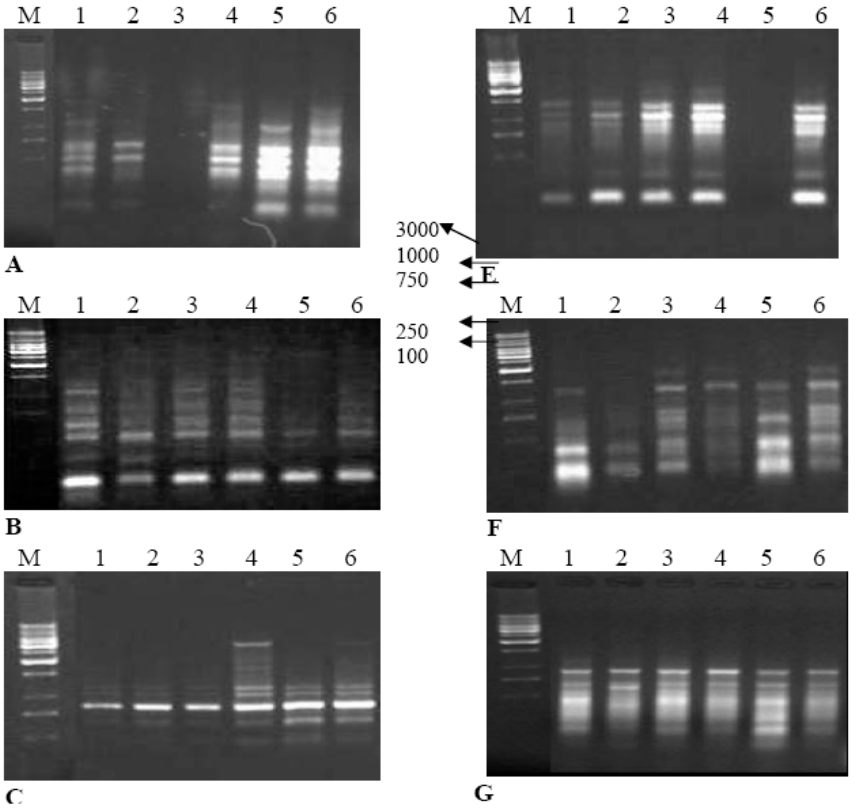

Figure 1. RAPD-PCR banding patterns of six *B. tabaci* host-associated populations by using seven random primers. **A**, Primer A9; **B**, Primer A12; **C**, Primer A15; **D**, Primer A18; **E**, Primer A19; **F**, Primer B3; **G**, Primer B7. **M**, DNA marker; **bp,** base pair **1**, eggplant; **2**, tomato; **3**, cotton; **4**, watermelon; **5**, squash; **6**, okra.

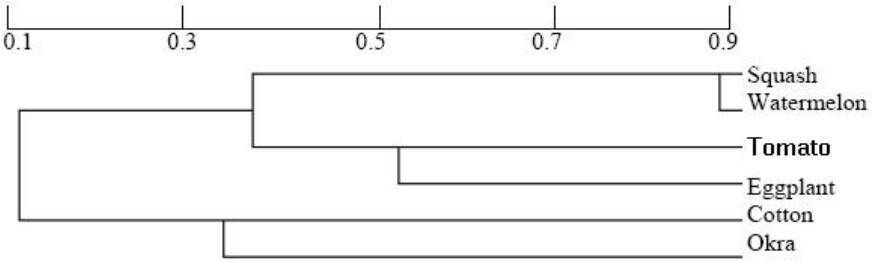

Figure 2. Phylogenetic relationships among different six host-associated populations of *B. tabaci* based on seven primers of RAPD-PCR.