EVIDENCE FOR PRESENCE MANY BIOTYPES OF *BEMISIA TABACI* (GENN.) WITHIN EGYPT

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Abstract

In an attempt to screen the primers to used in high taxonomic level of populations differences of *Bemisia tabaci* (Gennadius). Populations of *B.tabaci* were collected from five areas (Fayoum, Beheira, Gharbia, Ismailia and Beni-Suef) , within Egypt. The pooled DNA was studied using RAPD-PCR. OPA-2 primer gave unique profile for each sample in term of the migration of the RAPD bands and discriminated the populations of Gharbia, Beheira and Ismailia into three strains.

INTRODUCTION

The economic importance of whiteflies to Egyptian agriculture was recognized a long time ago (Priesner &Hosny,1932). The majority of agriculture in Egypt is located along the Nile Valley and in the Delta. These fertile soils and the mild climate are ideal for whitefly infestation where cotton, vegetables and fruits are produced (Idriss *et al.*1997). Moreover, the new reclaimed lands became invaded by the whitefly due to transference of transplantations and vegetables (Homam *et al.* 2003).

The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) exhibits a large amount of biological and genetic variation among natural populations (Brown *et al.* 1995b). The presence of biotypes or host races of this whitefly was first recognized in the 1950s, when it was discovered that morphologically indistinguishable populations of *B.tabaci* showed different biological traits with respect to host range, host-plant adaptability and plant virus-transmission capabilities (Bird 1957). Soon it was discovered that polymorphisms at the esterase locus were frequent and that those polymorphisms could be used as biotype markers (Costa & Brown,1991 Bedford *et al.* 1992, Wool *et al.* 1993 and Brown *et al.* 1995a) Up to the present time, approximately

19 distinct esterase phenotypeshave been characterized in populations of *B. tabaci* worldwide and named with a letter code from A to S. Recently Simon *et al.*(2003)recorded the biotype T in Italy on *Euphorbia characias* L. These letters have subsequently been used also to the respective biotypes (Bedford *et al.* 1992 , Brown *et al.*1995a and Banks *et al.* 1999). These biotypes can differ in multiple traits, such as host-plant adaptation, induction of phytotoxic reactions and insecticides resistance (Costa & Brown, 1991, Bedford *et al.* 1994). Most biotypes have a limited host and geographic rang, transmitting indigenous geminiviruses of local distribution and are of low agricultural importance. By contrast, the B biotype is highly polyphagous and has spread globally with the trade in ornamentals. This spread has made the movement of plant viruses into new areas easier with serious economic consequences (Bedford *et al.* 1994, Brown *et al.* 1996). These facts highlight the importance of assessing the biotype status of *B. tabaci* populations. The development of molecular markers based on nucleic acids technology has provided new insights in the study of *B. tabaci* variation. The random amplified polymorphic DNA technique (RAPD-PCR, Williams *et al.* 1990 and Welsh and McClelland 1990) is useful in differentiating biotypes (Perring *et al.* 1993 Guirao *et al.* 1997 and Homam *et al.* 2003).

MATERIALS AND METHODS

The samples of adult cotton whitefly ,*Bemisia tabaci* (Geen) were collected from cabbage from five areas (Fayoum, Beheria, Gharbia, Ismailia and Beni-Suef), Respectively Fig.1.

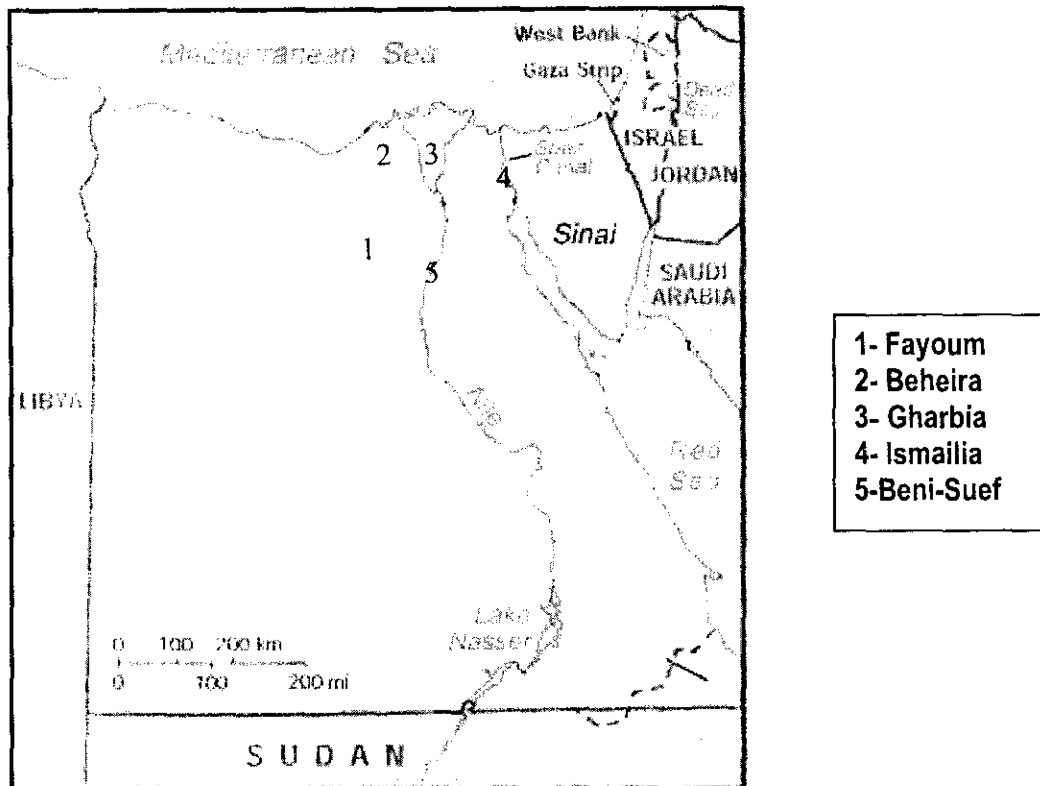

Fig 1.Map Showing The distribution of tested *Bemisia tabaci* populations within Egypt

Samples were deep frozen at -20ºC until use . Moreover also samples of immature stages (pupal case) were collected to be classified. DNA isolation was performed according to (Sambrook *et al.*1989) RAPD-PCR conditions: Genomic DNA was analyzed with RAPD–PCR technique according to the method described by Williams *et al.* (1990) &Welsh and McClelland (1990) .The primer used were 10- base long oligonucleotides of arbitrary sequence, which were obtained from Operon-A set (Operon, USA) , (OPA-2 and 20). In a 50 µl final volume PCR reaction (in a 0.25 Eppendorof tube), the components of the reaction mixture for each whitefly sample were 30 ng template DNA, 2µl dNTPs (10 mM), 5µl primer (5 Pico mole /µl), 5 µl $Mgcl_2$ (25 mM), 5µl Taq – polymerase buffer 10 X and 1.8 unit Taq- polymerase enzyme.

The temperature profiles were: Predenaturation at 95ºC for 6 minutes and denaturation, annealing, extension were repeated 40 cycles at 94ºC, 36ºC, 72ºC for 1,2 and 3 min., respectively. The post – extension lasted for 10 minutes at 72ºC.

Agarose gel electrophoresis: 25µl of PCR products of each samples were mixed with 5 µl of blue juice loading dye (6X), loaded on 1.8% agarose gel stained with 100µl of ethidium bromide (1000X) and subjected to electrophoresis at 50V for one hour. The PCR products were visualized on UV transilluminator, then photographed by using Polaroid MP4 camera. Statistical analysis: Data analyzed by similarity coefficient, S.Co. (Nei and Li ,1979).

RESULTS

Data concerning the DNA fingerprints or RAPD-PCR patterns of *B.tabaci* samples in five different areas (Fayoum, Beheira, Gharbia, Ismailia and Beni-Suef) were given in Table 1 as similarity coefficient. The characteristic patterns of the amplified PCR patterns using primer OPA-2 &20 were illustrated in Fig. 2.

Table 1. Similarity coefficient among five samples of *B.tabaci* adults

Governorates	Fayoum		Beheira		Gharbia		Ismailia		Beni-Suef	
Primers	OPA-2	OPA-20	OPA-2	OPA-20	OPA-2	OPA-20	OPA-2	OPA-20	OPA-2	OPA-20
Fayoum			0.13	0.30	0.40	0.73	0.22	0.30	0.25	0.20
Beheira					0.0	0.28	0.33	0.00	0.31	0.13
Gharbia							0.0	0.28	0.12	0.28
Ismailia									0.46	0.27
Beni-Suef										

Generally, it was obvious that ,the fingerprints which were generated by OPA-2 primer gave unique profiles for each samples in term of the number and migration of the RAPD bands.

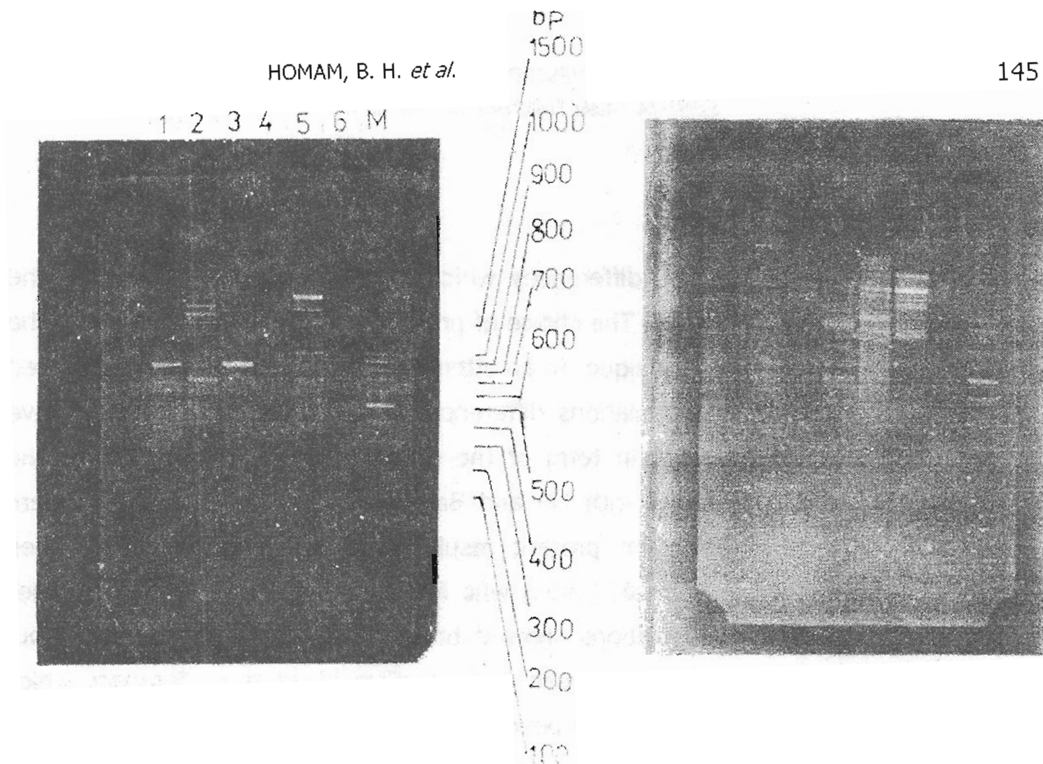

Fig 2. RAPD-PCR patterns using OPA-2 & 20 primer ,lane 1-5 samples from Fayoum, Beheira, Gharbia, Ismailia and Beni-Suef governorates, respectively, lane 6 without templet DNA, lane M molecular size marker

RAPD-marker by using OPA-2 primer was characterized by a low S.Co. among the five tested samples, the S.Co. of RAPD-marker among (Beheira & Gharbia) and

(Gharbia & Ismailia) were Zero. On the other hand, the highest S.Co. recorded moderate values (0.46 & 0.40) between (Ismailia & Beni-Suef) and (Fayoum & Gharbia), respectively.

The results clearly showed that, the samples of whiteflies varied in their products of RAPD-PCR in case of OPA-20. The obtained data illustrated that, the S.Co. between Fayoum and Gharbia had the highest value 0.73. On other hand, the lowest value of S.Co. between Beheira and Ismailia gave (0.0). On regarding the subsequent unlikeness among the tested population, adults of Beheira and Beni-Suef had S.Co. 0.13.

DISCUSSION

The apparent lack of even basic genetic information on *Bemisia tabaci* belies its importance as a major pest species. We used RAPD-PCR fingerprint of populations of *B.tabaci* (Fayoum, Beheria, Gharbia, Ismailia and Beni-Suef). This approach is a powerful means to study genetic variability of natural (Perring *et al.*1993 : Perring 1995, Guirao *et al.* 1997 and Homam *et al.* 2003). Samples of adult whitefly were collected from cabbage plant to ignore the host plant interference, moreover also samples of immature stages (pupal case) were collect and classified. The conventional classification result of all tested (immature stage) populations were *B.tabaci* .

PCR pattern illustrated the differences which have been observed during the biogeography survey of *B.tabaci*. The choice of primers is of major importance for the discriminatory power of the technique. In an attempt to screen the primers to be used in high taxonomic level of populations differences of *B.tabaci* OPA-2 primer gave unique profile for each sample in term of the migration of the RAPD bands. The present results run in common with Liu and Berry (1995) who described different utilities of different primers. The present result also agree with what has been reported by Bardakci and Skibinski (1994) who found that the pattern of similarities and differences between populations showed broad agreement across primers but overall level of similarity varied between primers. Five biotypes of *B.tabaci*, which morphologically indistinguishable, have been differentiated based on PCR pattern. This result agree with (Bedford *et al.*1994) where more than 10 biotypes were listed. The S.Co.between both Gharbia & Beheria, Gharbia & Ismailia recorded the lowest value (0.0). This mean that each populations of *B.tabaci* Gharbia, Beheria and Ismailia represents three strains according to Perring (1995) who analyzed the genomic DNA using PCR showed unique set of amplification products for the two strains (A&B). He analyzed those products for 3 populations of each strain indicated that populations shared 80-100% similarity ,but less than 10% similarity was observed between strains. The present results revealed that the sequences of OPA-2 may be conserved sequences which are most useful at higher taxonomic levels and in determining evolutionary relationships of *B. tabaci* populations. In this respect, Homam *et al.* (2003) mentioned that primer OPA-17 recorded 0.0 S.Co. between populations of each (Fayoum ,Beheira) & (Fayoum, Ismailia) & (Fayoum, Beni –Suef) & (Fayoum , Sohag). Also, He mentioned that, primer OPA -19 gave a S.Co (0.08) between Ismailia and Sohag. In spite of OPA-2 primer gave unique profile but gave resemble to which population have significant genetic similarity (Gharbia & Fayoum), (Beni Suef & Ismailia) were S.Co (0.40 & 0.46), respectively. This finding agrees with results of Homam *et al.* (2003) who mentioned that the RAPD –PCR patterns of pooled DNA of *B. tabaci* sampled from six area, (Fayoum, Gharbia, Beheria, Ismailia, Beni – Suef, and Sohag) grouped into two distinct clusters by Dendrogram analysis. The first comprised of whitefly collected from Gharbia and Fayoum, while the second comprised of Beheira, Ismailia, Beni-Suef and Sohag.

Also OPA-20 primer can be used in low taxonomic level among these populations.

It is essential to determine which biotypes or species are found in one specific area or different areas in order to use the most appropriate measures of control and help

in predicting possible outbreaks or detecting the source of viral infection (Bink-Moenen and Mound 1990). In addition adults fly mainly during day light hours (Bellow *et al.* 1988) and may be carried over long distance due to their minute size and transferring viruses between plants. To determine the invades of new biotypes for certain species we must compare between the old fingerprint and new fingerprint over interval times . For the previous we must start from now to use the molecular biology technique to prepare the fingerprints for the serious pests in Egypt.

CONCLUSIONS

The RAPD-PCR pattern of genetic variability of *B. tabaci* populations within Egypt indicated that OPA-2 primer can be discriminate the cryptic strains ,give unique patterns, used in high taxonomic level and evidence for presence many or numerous biotypes related to biogeographic area within Egypt.

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دليل على وجود عديد من Biotypes لذبابة القطن البيضاء بيميسيا تباسي (جن) داخل مصر.

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مركز البحوث الزراعية – معهد بحوث وقاية النباتات – الدقي – جيزة – مصر

فى محاولة لعمل غربلة للبادئات المراد استخدامها في مستوى تصنيفي عالي لعشائر مختلفة من ذبابة القطن البيضاء بيميسيا تباسي . العشائر المختبرة جمعت من خمسة مناطق هي (الفيوم، البحيرة، الغربية، الإسماعيلية، وبني سويف) على الترتيب داخل مصر. درس DNA باستخدام RAPD-PCR، وجد أن البادئة OPA-2 أعطت صور منفصلة لكل عينة من حيث حزمة RAPD المهاجرة من خلال جهاز الفصل الكهربي، أيضا النتائج أعطت دليل على وجود عديد من biotypes مرتبطا بالتوزيع الجغرافي لهذه الآفة في مصر.