

Host associated genetic variations in whitefly, *Bemisia tabaci* (Genn.)

R K Sharma, V K Gupta*, J Jindal and V K Dilawari

Insect Molecular Biology Lab, Department of Entomology, Punjab Agricultural University, Ludhiana 141 004, India

Received 18 May 2007 ; revised 20 December 2007; accepted 1 March 2008

Genetic variability due to host plants was studied in whitefly, *Bemisia tabaci* (Genn.), populations that were collected from fields of different crops (cotton, brinjal, potato, tomato and soyabean) and a weed (*Sida* sp.), and maintained on their respective host plants for 12 generations. Comparative RAPD-PCR analysis of these populations led to identification of 85 different polymorphic bands or host specific markers. Of these, 39 markers were identified for single-host specificity; maximum markers were identified for tomato (14) and cotton (13); followed by brinjal (5), *Sida* sp. (4) and soyabean populations of whitefly (3). Similarly, of 23 two-host specific markers, maximum markers were identified in cotton (10) and tomato (11) whitefly. This is the first report establishing the existence of host-plant specific genotypes in *B. tabaci* that is likely to have broad impact on its pest status and vectoring ability for different geminiviral diseases. The genetic similarity dendrogram based upon the comparative RAPD profiles showed the existence of a high level of genetic relatedness (72-85%) amongst the investigated whitefly types and the lineage of their origin from a common type. This lineage suggests that the whitefly types holding specificity for different host plants under study have evolved as three distinct genetic groups formed by cotton, *Sida* and soyabean (Group 1); potato and brinjal (Group 2); and tomato (Group 3).

Keywords: *Bemisia tabaci*, genotype analysis, genetic similarity, RAPD markers

Introduction

The tobacco whitefly, *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae), is known by several common names, e.g., tobacco, cotton or sweet potato whitefly. Being polyphagous, *B. tabaci* has been reported on more than 600 host plants, most of which are of immense importance to worldwide agricultural production¹. *B. tabaci* has emerged as a serious pest of cotton and many vegetable crops, not only due to direct losses caused by it but also by being vector of geminiviral diseases of these crops. Whitefly remains active on many hosts throughout the year. Of these, brinjal, chillies, cucurbits, cotton, mentha, okra, potato, rapeseed, sunflower, soyabean and tomato are important hosts for this pest. Besides, several weeds, such as *Sida* sp., *Abutilon indicum* and *Althea rosea*, have also been identified to serve as collateral hosts to whitefly in Punjab². Since different host crops serve as alternate host to whitefly, they are likely to have a strong influence on selection of specific whitefly genotypes out of the existing population in a particular agro-climatic region. Studies on host related phenotypic variations leading to biotypes had concluded that taxonomic characters vary widely not

only among the populations in different hosts but also among the different varieties of the same host³⁻⁵. Allozyme and microsatellite marker analyses of pea aphid (*Acyrtosiphon pisum*) populations from pea, clover and alfalfa has established the existence of genetically divergent distinct host races⁶. Such ecologically and genetically distinct host races offer an ideal biological system for studies on sympatric speciation⁷. The elucidation of host specificity in *B. tabaci* populations proliferating on different host crops in the same geographical would provide an insight into mechanisms involved in evolution of new races/biotypes in this agronomically important pest species. In view of the above, the present study was undertaken to identify existence of host correlated genetic variability amongst *B. tabaci* populations from six different host plant species using RAPD-markers.

Materials and Methods

Collection of Samples

Whitefly populations were collected from potato, tomato, brinjal, soyabean and cotton fields of Punjab Agricultural University, Ludhiana, and from a weed *Sida* sp. growing wildly in the University campus in the Kharif season during the month of August-September 2005. Subsequently, all individual

*Author for correspondence:

Tel: 91-161-2401960 Ext 320; Fax: 91-161-2400945

E-mail: virashkgupta@gmail.com

populations were maintained continuously for 12 generations on the respective host plants that were grown under isolated screen house conditions. The purity of all 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. For molecular analysis, samples of 10-15 whitefly individuals (adults) from 10th, 11th and 12th generations were collected from respective population and stored in 70% ethanol.

Extraction and Quantification of Whitefly DNA

Total DNA from whitefly individuals was isolated by the method of DeBarro and Driver⁸. Briefly, a single whitefly was thoroughly macerated with a micropestle in a 1.5 mL Eppendorf tube containing 50 μ L lysis buffer containing 50 mM KCl, 10 mM Tris-Cl pH 8.4, 60 μ g mL⁻¹ proteinase K (Merck, >30 m Anson units/mg), 0.45% Nonidet NP-40 and 0.45% Tween 20. The macerated suspension was given heat treatment at 65°C for 45 min followed by 95°C for 10 min, and centrifugation at 13.2 kg for 3 min. Supernatant containing extracted DNA was diluted with equal volume of Milli-Q quality autoclaved water, and stored at 20° C until used. The quality and concentration of DNA (~10 ng/ μ L) was assessed by agarose gel electrophoresis (0.7% in TAE buffer) and UV spectrophotometry (A_{280} , A_{280}/A_{260} ratio).

RAPD-PCR Analysis

Comparative RAPD-PCR analysis of whitefly from different hosts was performed using whitefly DNA as template and different RAPD primers (OPERON Technologies Inc, Alameda, California) for PCR amplification. PCR reactions were performed in 25 μ L reaction mixtures, each containing 20 ng template DNA solution (2 μ L), 1 mM dNTPs mix (5 μ L), 10 μ M RAPD primer (5 μ L), 2.0 U Taq Polymerase (MBI, Fermentas) and 1.5 mM MgCl₂ in 1X Taq reaction buffer. The PCR amplification programme consisted of 95°C for 5 min (preheating), 95°C for 1 min, 40°C for 1 min, 72°C for 2 min (36 cycles), 72°C for 10 min (final extension) and stored at 4°C until used. The amplified DNA products were separated by electrophoresis along with a MW marker (100 bp ladder plus, MBI Fermentas) using 1.5% agarose gel in TAE. The gel was stained with ethidium bromide and the banding profiles visualized and photographed using UV-Gel Documentation system (UltraLum).

Analysis of RAPD-PCR Profiles for Genetic Relatedness

All the individual bands of different host specific whitefly samples in RAPD banding profile were scored for presence (1) or absence (0) for all the individual population. The data matrix was used to calculate Jaccard's coefficient and a genetic relatedness dendrogram was constructed using 'SimQual' function of the UPGMA program of the software NTSYS pc version 2.02e⁹.

Results and Discussion

Identification of Genetically Inheritable Host-Plant Specific Marker Bands in RAPD-PCR Profiles

Identification and validation of molecular marker is the first step towards understanding genetic diversity within an insect population, if possible, to differentiate them into definite biotype or races. Against specific molecular markers, such as microsatellites, which identify genetic variability based upon limited number of genetic regions, RAPD profiles generated with a number of RAPD primers are considered advantageous as they help to generate comparative profiles based on randomly chosen genetic regions all over the genetic element, DNA. In *Bemisia*, RAPD markers have been used for analysis of genetic variation^{8,10-12}, taxonomic studies¹³, biotype identification and geographical distribution^{14,15}. Therefore, in order to select genetically heritable markers, each individual whitefly population was sampled for three successive generations (10th, 11th & 12th) to identify only genetically inheritable bands/markers in the RAPD profiles. Thus, comparative RAPD profiles were generated by using single individual from each of three successive generations for all the six host specific populations and 24 different RAPD primers. Of these, only nine primers (B-05, B-10, B-20, C-01, C-03, C-04, C-07, F-03, H-16) those amplified distinct polymorphic bands were used for studying genetic variability among different host specific populations. With these nine primers, matching RAPD profiles were consistently amplified in whitefly samples from all three successive generations of a single host type establishing the stable heritability of a number of RAPD marker bands in these profiles (Fig. 1). The comparative analysis of these RAPD profiles from different whitefly types resulted in identification of a number of polymorphic markers holding specificity for one or more hosts. These genetic differences visualized as host specific polymorphic bands, amplified from particular host specific whitefly,

represented host specific molecular markers for respective whitefly genotype (Table 1). It was noted that where some of the molecular markers were

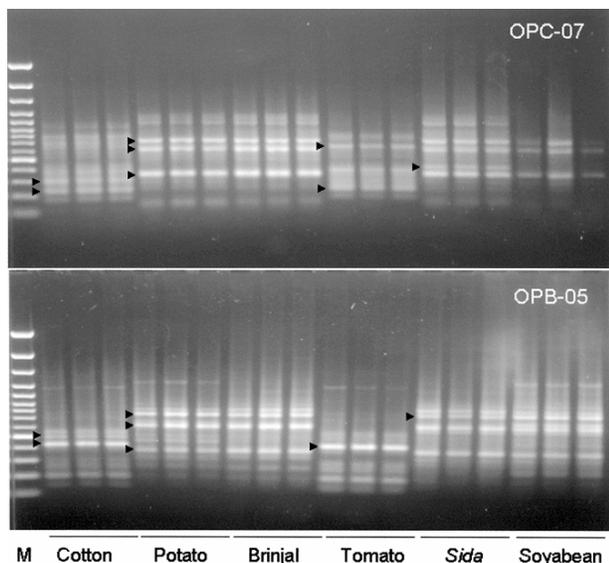


Fig. 1—Comparative RAPD profile of different host specific whitefly populations with two primers (OPC-07 and OPB-05) showing genetically stable markers (▶); M, 100 bp DNA size maker lane (MBI Fermentas). (Under each host crop the three lanes (from left to right) represent whitefly individual from 10th, 11th and 12th generation)

amplified in more than one type of host specific whitefly, others were amplified only in a single whitefly type holding specificity for a single host plant. In total 85 different markers were identified (Table 2). Of these, 39 markers were specific for a single-host; the maximum single-host markers were identified for tomato (14) and cotton (13), followed by brinjal (5), *Sida* sp. (4) and soyabean (3). However, no single-host specificity marker was identified for potato. Similarly, of 23 two-host specific markers identified, 10 and 11 markers were identified in cotton and tomato, respectively; while 5-8 markers were identified to whitefly type holding specificity to other four hosts. Besides, 10 additional markers were identified, which were uniquely shared by potato, brinjal, *Sida* sp. and soyabean.

In the present study, the host specific genetic diversity was identified in *B. tabaci*, a polyphagous insect. The knowledge of genetic diversity holds significance in identification of 'specialist' genotypes present on specific hosts, or 'generalist' genotypes colonizing several host types including cultivated crops or wildy growing common weeds. As the whitefly 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

Table 1— RAPD molecular markers associated with host specificity in whitefly

Host crop	RAPD-PCR marker bands		Total #
	Single-host specificity	Multiple-host specificity	
Cotton	B-05 ₄₁₀ , B-05 ₅₀₀ , B-05 ₅₈₀ , B-10 ₄₀₀ , B-10 ₇₅₀ , C-01 ₃₅₀ , C-03 ₃₅₀ , C-04 ₅₁₀ , C-04 ₇₂₀ , C-07 ₃₀₀ , C-03 ₇₀₀ , C-07 ₈₀₀ , H-16 ₅₅₀	B-05 ₄₇₀ , B-05 ₃₂₅ , B-10 ₅₂₅ , B-20 ₅₃₀ , B-20 ₆₈₀ , C-01 ₆₆₀ , C-01 ₆₅₀ , C-04 ₄₉₀ , C-04 ₆₀₀ , C-07 ₉₀₀ , F-03 ₈₀₀ , F-03 ₉₀₀ , H-16 ₄₇₀ , H-16 ₅₉₀	27
Potato	None	B-05 ₃₂₅ , B-05 ₃₉₀ , B-05 ₅₅₀ , B-05 ₇₅₀ , B-10 ₄₆₀ , B-10 ₅₉₀ , B-10 ₇₀₀ , B-20 ₅₃₀ , B-20 ₆₈₀ , B-20 ₉₈₀ , C-01 ₄₃₀ , C-03 ₄₈₀ , C-03 ₆₅₀ , C-03 ₁₀₅₀ , C-04 ₃₄₀ , C-04 ₆₈₀ , C-07 ₃₉₀ , C-07 ₅₀₀ , C-07 ₇₂₀ , F-03 ₆₀₀ , H-16 ₅₀₀ , H-16 ₇₀₀ , H-16 ₇₄₀ , H-16 ₉₅₀	24
Brinjal	B-05 ₆₈₀ , B-20 ₄₄₀ , C-07 ₅₂₅ , C-01 ₉₅₀ , C-03 ₄₆₀	B-05 ₃₉₀ , B-05 ₅₅₀ , B-05 ₆₀₀ , B-05 ₇₅₀ , B-05 ₈₁₀ , B-10 ₄₉₀ , B-10 ₇₀₀ , B-20 ₅₃₀ , B-20 ₆₈₀ , B-20 ₉₈₀ , C-03 ₁₀₅₀ , C-01 ₄₃₀ , C-01 ₄₇₀ , C-03 ₄₈₀ , C-04 ₃₄₀ , C-04 ₄₂₀ , C-04 ₆₈₀ , C-07 ₅₀₀ , C-07 ₇₂₀ , F-03 ₆₀₀ , F-03 ₈₀₀ , F-03 ₉₀₀ , F-03 ₉₅₀ , H-16 ₅₉₀ , H-16 ₇₀₀ , H-16 ₇₄₀ , H-16 ₉₅₀	32
Tomato	B-05 ₄₂₀ , B-05 ₇₂₀ , B-20 ₄₈₀ , B-20 ₆₀₀ , B-20 ₆₄₀ , C-01 ₄₀₀ , C-01 ₄₅₀ , C-01 ₅₀₀ , C-01 ₇₀₀ , C-03 ₈₀₀ , C-07 ₄₁₀ , C-07 ₅₅₀ , C-07 ₆₇₀ , F-03 ₃₀₀	B-05 ₃₀₀ , B-05 ₄₇₀ , B-10 ₅₂₅ , C-01 ₆₅₀ , C-01 ₆₆₀ , C-04 ₄₂₀ , C-04 ₄₉₀ , C-04 ₆₀₀ , C-07 ₅₀₀ , C-07 ₉₀₀ , F-03 ₅₀₀ , F-03 ₈₀₀ , F-03 ₉₅₀ , H-16 ₅₀₀ , H-16 ₄₇₀ , B-05 ₆₀₀	30
<i>Sida</i>	B-05 ₈₅₀ , C-03 ₅₂₀ , C-07 ₄₃₀ , H-16 ₆₈₀	B-05 ₃₀₀ , B-05 ₃₉₀ , B-05 ₅₅₀ , B-05 ₆₀₀ , B-05 ₇₅₀ , B-05 ₈₁₀ , B-10 ₅₉₀ , B-10 ₇₀₀ , C-01 ₄₀₀ , C-01 ₄₃₀ , C-01 ₄₇₀ , C-03 ₆₅₀ , C-04 ₃₄₀ , C-04 ₃₉₀ , C-07 ₃₉₀ , C-07 ₇₂₀ , F-03 ₆₀₀ , H-16 ₇₄₀ , H-16 ₉₅₀ , H-16 ₉₇₀	24
Soyabean	C-03 ₁₀₀₀ , H-16 ₃₈₀ , H-16 ₄₀₀	B-05 ₃₀₀ , B-05 ₃₉₀ , B-05 ₅₅₀ , B-05 ₆₀₀ , B-05 ₇₅₀ , B-10 ₄₆₀ , B-10 ₄₉₀ , B-10 ₅₉₀ , B-10 ₇₀₀ , B-20 ₉₈₀ , C-04 ₃₉₀ , C-04 ₆₈₀ , C-01 ₆₆₀ , C-01 ₄₇₀ , C-01 ₄₃₀ , C-01 ₄₀₀ , C-07 ₃₉₀ , C-07 ₇₂₀ , F-03 ₅₀₀ , F-03 ₆₀₀ , H-16 ₇₄₀ , H-16 ₉₅₀ , H-16 ₉₇₀	26

Table 2—Distribution of different host specific markers in whitefly with respect to their host specificity

Host specificity of marker	Total no. of markers	Host crop					
		Cotton	Potato	Brinjal	Tomato	<i>Sida</i> sp.	Soyabean
Single-host	39	13	0	5	14	4	3
Two-hosts	23	10	6	8	11	5	6
Three-hosts	13	4	8	9	5	6	7
Four-hosts	10	0	10	10	0	9	10
Total	85	27	24	32	30	24	26

example of the evolutionary transition leading to sympatric speciation in *B. tabaci*¹⁶. The sequence information on these RAPD-DNA markers can be used to design more robust, specific molecular markers for use in molecular tracking of different whitefly types with specificity to different host plants.

Genetic Relatedness amongst Different Host Plant Specific Whitefly Populations

Computation of comparative amplification data with nine primers for genetic relatedness amongst whitefly from different hosts using NTSYS resulted in a genetic similarity dendrogram, which supported the existence of high level of genetic relatedness amongst the investigated whitefly types and the lineage of their origin from a common type (Fig. 2). In this lineage, the whitefly types under study appeared to have developed as three distinct plant host genetic groups formed by cotton, *Sida* and soyabean (Group 1); potato and brinjal (Group 2); and Tomato (Group 3), with genetic similarity (relatedness) of 87.5, 76 and 80% amongst the host group members. In this respect, whitefly from tomato although shares a position close to whitefly from potato and brinjal, it appears to be the common ancestor for all other whitefly types and, hence, been assigned a different group in this genetic similarity dendrogram. The results establish a definite but undefined host specific pressure that selects definite genetic variants from many which exist in the field populations of whitefly or emerge due to above pressure from the preexisting genotype(s). Following the analysis, more number of whitefly types from other host plant can be assigned to a particular host group or to another new group. Further, it would be worth looking on the differences seen among the populations presently studied for whether the molecular differences would be substantiated by virus transmission characters. The identified host specificity in *B. tabaci* is interesting and it requires further validation with populations collected from

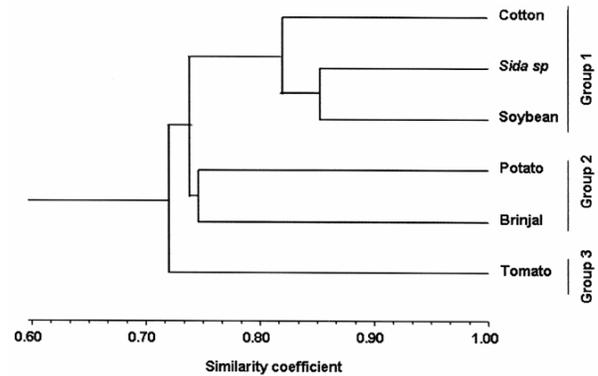


Fig. 2—Dendrogram showing genetic relatedness amongst whitefly populations from different host crops

different geographic regions. These markers after sequencing can form the basis for development of more specific markers. The application of which will lead to better understanding on the genetic diversity and to differentiate the whitefly populations on the basis of their host relationships and, hence, their efficient management. The present study highlights the importance of understanding not only the demographic parameters to genetic diversity, but also the more intricate correlation of genetic diversity to host types in agro-ecosystems. Such studies assume significance in the wake of evidence that whitefly from each host is not equally efficient as vector of cotton leaf curl virus (CLCuV) and other geminiviruses¹⁷.

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