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SCAR markers for identification of host plant specificity in whitefly, *Bemisia tabaci* (Genn.)

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RAPD markers (8 in number), which identified specificity in *Bemisia tabaci* (Genn.) to a single or two different host plants (out of cotton, brinjal, tomato, soybean and *Sida* sp., a weed) were sequenced. Based upon these nucleotide sequences, 8 sets of SCAR primers were developed for PCR amplification of the respective SCAR markers from *B. tabaci* genotype holding specificity to a specific host plant. During evaluation, these primers amplified SCAR markers of expected sizes as per position of primer sequences in the determined sequence for the respective RAPD marker and only from the specific *B. tabaci* genotype. Subsequent validation of each of these SCAR markers with 10 individuals from a freshly collected *B. tabaci* population from the respective field of above field crops (and potato), and wildly growing *Sida* sp. plants, resulted in correct amplifications with majority of the whitefly individuals (6-8), while the specific SCAR marker was not amplified with a small fraction of *B. tabaci* individuals (2-4). Thus, the SCAR markers were found to be true to their identity, whereas failure to amplify the same from minor fraction of the field population could be due to mixed population effect, wherein the field population gets mixed with whitefly from alternate source crops and remained undetected. The finding that majority of the individuals being detected by respective marker supported the specificity of developed SCAR markers for molecular monitoring of host specific *B. tabaci* individuals and populations, besides establishing the existence of host selection of whitefly by the plant host type in nature.

Keywords: *Bemisia tabaci*, host plant specificity, SCAR marker

Introduction

Bemisia tabaci (Genn.) (Hemiptera: Aleyrodidae), commonly known as sweet potato whitefly, is an insect vector of almost all the geminiviruses. It has emerged as one of the most devastating agricultural pests worldwide, feeding on an estimated 900 plant hosts, most of which are of immense worldwide importance to agricultural production[1-3]. Many studies on biochemical and molecular analysis on *B. tabaci* collected from different geographical regions of the world has established that *B. tabaci* is a species or biotype complex of highly cryptic sibling species, which lack morphological traits for differentiation of behavioral and/or genetic variants[4-10]. Recently, well-resolved molecular phylogeny of *B. tabaci* specimens, based upon microsatellite, mitochondrial and ribosomal markers, has identified a large number of races and genetic groups that has established in different parts of the world with each one represented by a large number of genetic populations and subpopulations with little or no gene flow between them[11].

In a specific geographic region, native whitefly remains active on many cultivated plant as hosts and on weeds as collateral hosts[12] throughout the year. These host plants serving as alternate hosts to whitefly are known to exert a strong influence on selection of specific whitefly genotypes out of the existing genotypes in a particular agro-climatic region[13-14]. Studies on host related variations leading to biotypes had also concluded that taxonomic characters vary widely not only among the *B. tabaci* populations on different hosts but also among the different varieties of the same host[15-18]. Such ecologically and genetically distinct host races offer an ideal biological system for studies on sympatric speciation[19].

Molecular markers based upon established mitochondrial, ribosomal and microsatellites have variously proved useful in genetic differentiation amongst inter and intraspecific variants in different insect species that have established in different agroclimatic regions[11]. Adaptation of a specific insect

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species to different type of local pressures, including plant host differences, results from a number of unknown genetic modifications that are randomly distributed all over the insect genome and may not be mapped in the established regions to escape notice. As against specific molecular markers, such as, microsatellites, which identify genetic variability based upon limited number of genetic regions, RAPD profiles are considered advantageous as they help to generate comparative profiles based on randomly chosen genetic regions all over the genome[20-21]. In *Bemisia,* RAPD markers have been used for analysis of genetic variation[4,19,22,23], taxonomic studies[10], biotype identification and geographical distribution[24,25].

Authors have earlier reported the existence of plant host specificity in whitefly populations collected from fields of six different plant hosts through identification of 85 genetically stable (inheritable) RAPD markers[26]. Biologically, these whitefly populations expressed differing efficiencies both for the acquisition as well as transmission of cotton leaf curl virus[26]. However, reproduction of RAPD markers, which though has been proved useful for phylogenetic analysis of various insect species[4,19,22,23], often suffer from lack of specificity and sensitivity to the reaction conditions[20]. More recently, robust SCAR markers that were designed based upon the nucleotide sequence of specific RAPD-PCR markers were found stable, easy to use and inexpensive for single locus assay in different crops[27-30], and also in specific identification of *B. tabaci* biotypes[31,32]. Therefore, in order to develop specific molecular diagnostic markers, authors report on the conversion of 8 pre-identified RAPD markers[26] for molecular identification of restricted plant host specificity in *B. tabaci* as well as their evaluation and validation.

Materials and Methods

Plant Host Specific Whitefly Population

Individual whitefly populations collected earlier from potato, tomato, brinjal, soybean and cotton fields, and from a weed *Sida* sp., were subsequently maintained continuously on their respective host plants under isolated screen house conditions[26]. For molecular analysis, samples of 10-15 whitefly individuals (adults) were collected from the 14th generation of respective population and stored in 70% ethanol. Besides, fresh populations from respective crops, or wildly growing *Sida* sp., were also collected from fields of Punjab Agricultural University, Ludhiana in the months of August, 2007 to December, 2007.

Isolation of Whitefly DNA

Total DNA from whitefly individuals was isolated by a standard methods[19]. A single whitefly was macerated with a micropestle in a 1.5 mL Eppendorf tube containing 50 µL lysis buffer (50 m*M* KCl, 10 m*M* Tris-Cl, *p*H 8.4), 60 µg/mL 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 65ºC for 45 min, followed by 95ºC for 10 min and centrifuged at 13 kg for 3 min. Supernatant containing extracted DNA was diluted with equal volume of Milli-Q quality autoclaved water, and stored at –20ºC until used. The quality and concentration of DNA (~10 ng/µL) was assessed by agarose gel electrophoresis (0.7% in TAE buffer) and UV spectrophotometry (A280, A280/A260 ratio).

Source of RAPD Marker DNA

Eight RAPD markers that have shown plant host specificity in *B. tabaci* against a single or two plant hosts were selected from our previous studies[26]. These markers along with their size, plant host specificity and GenBank acc. no. of the respective nucleotide sequence, as determined in the present study, are described in Table 1.

Cloning and Sequencing of RAPD Marker DNA

RAPD-marker DNA was purified from the excised agarose blocks with a selected RAPD marker band using Gel extraction kit QIAEX II (QIAGEN,

Table 1—Plant host specific RAPD markers in whitefly with respective plant host specificity

RAPD marker		GenBank	Expected host
Marker *	Size (bp)**	Acc. no.	specificity
B-05$_{410}$	415	GQ373184	Tomato
C-03$_{460}$	469	GQ373185	Brinjal
C-03$_{350}$	354	GQ373186	Cotton
H-16$_{380}$	385	GQ369451	Soybean
C-04$_{390}$	396	GQ369452	*Sida* sp. & Soybean
B-10$_{400}$	403	GQ373187	Cotton
C-04$_{420}$	430	GQ369453	Brinjal & Tomato
B-10$_{525}$	530	GQ373188	Cotton & Tomato

* As per Sharma *et al* [26],
** As per nucleotide sequence of respective RAPD marker

Germany). The purified RAPD-DNA fragments were ligated into a PCR cloning vector pTZ57R/T (*Eco*R5) using InsT/Aclone PCR Product cloning kit (MBI Fermentas, USA) and the ligation product transformed into *Escherichia coli* DH5-α competent cells. The recombinant clones were selected on Luria broth agar (LB-agar) supplemented with ampicillin (100 μg/mL) and X–Gal/IPTG. From overnight culture of a recombinant clone, the recombinant plasmid with cloned band-DNA was purified by GeneElute Plasmid MiniPrep kit (Sigma). Both the strands of the cloned band-DNA in respective recombinant plasmid were custom sequenced by 'Sequencing Service' of Bangalore Genei, Bangalore, India using universal M13F and M13R primers, the annealing sites which bordered the multiple cloning site in the cloning vector. The sequence of cloned RAPD-DNA was identified as the sequence that was internal (3' sides) to the respective RAPD primer sequences that were located on the 5' ends of each of the two complementary strands of the cloned DNA.

Designing of SCAR Primers

Based on the sequence of the individual cloned product (RAPD-marker), pairs of compatible SCAR primers were designed in 5' regions, using the software program FastPCR for Windows from PCR-Team (University of Helsinki, Finland), for to be used in specific identification of plant host specific loci in specific whitefly genotypes that were earlier identified by RAPD markers[26]. These primers were synthesized by custom service of Q-Biogenes Research Services, France.

PCR Reaction and SCAR Amplifications

PCR reactions were performed in 25 μL reaction mixtures, each containing ~20 ng template DNA solution (2 μL), 1 m*M* dNTPs mix (5 μL), 1 μL each of the forward and reverse SCAR primers (10 μ*M*), 2.0 U Taq Polymerase (MBI, Fermentas) and 1.5 m*M* $MgCl_2$ in 1× Taq reaction buffer. The PCR amplify-cation programme consisted of 95ºC for 5 min (preheating), 95ºC for 1 min, 58ºC for 1 min, 72ºC for 2 min (36 cycles), 72ºC for 10 min (final extension) and stored at 4ºC until used. PCR products were run on a 0.7 % (w/v) agarose gel in 1× TAE and gel stained in ethidium bromide. Presence or absence of the SCAR band was visually scored and its size determined by a standard DNA marker which was co-run with the amplified DNA samples and its pattern recorded on Gel documentation system.

Results and Discussion

Selection of Genetically Inheritable Plant Host Specific RAPD Marker for Conversion into SCAR Marker

Earlier, authors had identified 85 genetically stable (inheritable) RAPD markers that held specificity for a single- or multiple-host plant specificity out of the six host plants studied (cotton, soybean, brinjal, tomato, potato and *Sida* sp.[26]). These markers represented the existence of unique DNA regions that possibly afforded specificity for respective plant host. From diagnostic point of view only those markers that expressed high level of specificity in whitefly were considered to be of practical significance. Therefore, for conversion into SCAR markers, only those RAPD markers were selected which held specificity to a single plant host ($B\text{-}05_{410}$, $B\text{-}10_{400}$ & $C\text{-}03_{350}$ in cotton whitefly; $C\text{-}03_{460}$ in brinjal whitefly; & $H16_{380}$ in soybean whitefly) or two different plant hosts ($B\text{-}10_{525}$ in cotton & tomato whitefly; $C\text{-}04_{420}$ in brinjal & tomato whitefly; & $C\text{-}04_{390}$ in *Sida* sp. & soybean whitefly) (Table 1).

Nucleotide Sequence of RAPD Markers and Designing of SCAR Primers

The nucleotide sequence of different RAPD markers as determined using universal M_{13} primers through Custom sequencing was processed, submitted to GenBank and assigned GenBank acc. no. (Table 1). Based on individual sequences, SCAR primer pairs were designed and the details on primer sequences, position in respective RAPD marker sequence, annealing temperature and expected size of SCAR marker are given in Table 2. While designing primers, Fast PCR program provided specific annealing temperatures for respective SCAR primer pairs; however, a single annealing temperature (58ºC) that supported good amplification by all SCAR primer sets from respective whitefly type was found suitable as determined by performing test PCR amplifications in a 'Master gradient thermocycler' (Eppendorf) at different annealing temperatures (data not given).

Evaluation of SCAR Primers for Specificity

Specific PCR amplification were performed using respective SCAR primers with total DNA isolated from a pooled sample of 10 whitefly individuals, collected from screen house populations being maintained on respective host plants, and the amplification profile is given in Fig. 1 (A-H). These amplification profiles using DNA from six different host plant specific whitefly types showed that all the

Table 2—Different SCAR primers designed for specific amplification of plant host specific SCAR markers from whitefly

Marker		SCAR Primers			Ann. temp**	Expected size of
RAPD	SCAR		Sequence 5'→3'	Position*	ºC	SCAR marker (bp)
$B\text{-}05_{410}$	$ToB05._{396}$	F	TGCGCCCTTCAAACGACGCCA	1-21	58.4	396
		R	CAGCTTGTTAACGTCATGGTGG	396-375		
$C\text{-}03_{460}$	$BrC03._{445}$	F	CAGATTCGTGATTAGCATTAGGA	11-33	54.2	445
		R	CACGAAGTATAACGGCTTCGACA	455-433		
$C\text{-}03_{350}$	$CoC03._{324}$	F	TTCCGTAGATATCAGGACTGCG	10-32	57.3	324
		R	ATTACACATGATCGATCGACG	334-314		
$H\text{-}16_{380}$	$SoH16._{348}$	F	TCTCAGCTGGATTCGAGCTGTGG	1-23	58.6	348
		R	TGACCATGATTACGCCAAGCTC	348-327		
$C\text{-}04_{390}$	$SiSoC04._{380}$	F	CCGCATCTACATTCGGAGCTC	1-21	57.2	380
		R	ATATGACTGCCTCTCACTCTCTT	380-358		
$B\text{-}10_{400}$	$CoB10._{373}$	F	CCCTGATCACCATCGAACCATCG	10-32	58.8	373
		R	TATGCTGTTAGCGTTCCTGACC	382-360		
$C\text{-}04_{420}$	$BrToC04._{326}$	F	AGGTTTAAGCACGATCTCGGCCT	43-65	59.0	326
		R	CATCGATGGTGAAAGTGTAGGAT	368-346		
$B\text{-}10_{525}$	$CoToB10._{471}$	F	AGGTTTAAGCACGATCTCGGCCT	28-50	57.5	471
		R	TCGGATTGCCTCAACAGCTAAAT	498-476		

*Base number in respective RAPD marker sequence; ** Values as determined using Fast-PCR program.

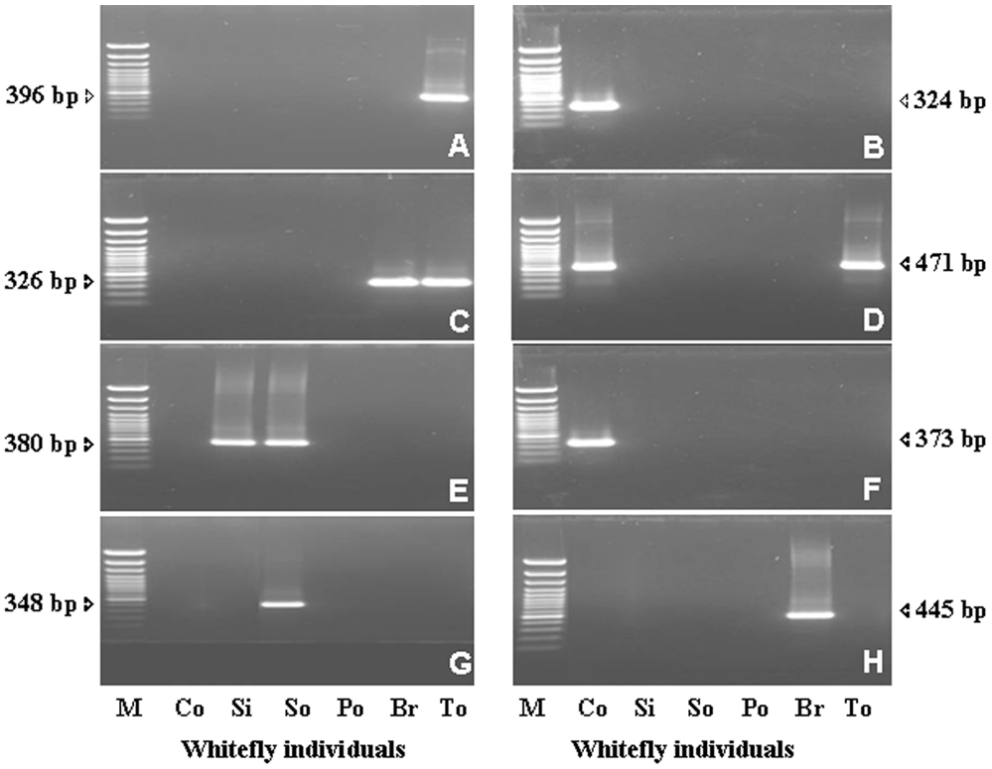

Fig. 1—Testing and evaluation of SCAR primers for plant host specific markers in whitefly. Host plants: Co-Cotton, Si-*Sida*, So-Soybean, Po- Potato, Br- Brinjal, To- Tomato. Scar Primer: A- ToB05396, B- CoC03324, C- BrToC04326, D- CoToB10471, E-SiC04380, F- CoB10373, G- SoH16348, H- BrC03445, & M- 100 bp DNA size marker lane (MBI Fermentas).

8 SCAR primer sets supported the amplification of single DNA product of expected size (Table 2) from the whitefly types holding respective host plant specificity only. Thus, all the 8 SCAR primer sets proved true to their specificity for respective whitefly types. Genetically all these whitefly genotypes though related to each other by more than 72% (UPGMA based genetic similarity index[26]), the sufficient genetic differences (28%) among these whitefly genotypes support diagnostically important differentiation through developed SCAR markers. SCAR markers common for brinjal and tomato (BrToC04.326), cotton and tomato (CoToB10.471) and *Sida* sp. and soybean (SiC04.380) most likely have their locations in genomic DNA regions that are shared by them but do not exist in other whitefly types. None of these SCAR marker were amplified from whitefly genotype specific to potato (for which no suitable RAPD marker was available for conversion into SCAR), which further supports the high specificity of the same.

Validation of SCAR Primers with Field Collected Whitefly Populations

The amplification of SCAR markers with respective SCAR primer set (s) was attempted with whitefly individuals collected from fresh whitefly populations of respective host plants from the fields of respective host plants (August-December, 2007). Total DNA from 10 whitefly individuals from respective whitefly types was used as template for PCR amplification with respective SCAR primer sets (Table 3) and the profiles are given in Figs 2 and 3.

Table 3—No. of whitefly individuals detected by SCAR markers in whitefly populations from fields of different plant hosts

SCAR marker	Plant host	No. of whitefly individuals showing specific amplification*	Fig.
$ToB05_{396}$	Tomato	6	2-D
$BrC03_{445}$	Brinjal	7	2-E
$CoC03_{324}$	Cotton	7	2-A
$SoH16_{348}$	Soybean	7	2-H
$CoB10_{373}$	Cotton	7	2-B
$SiSoC04_{380}$	*Sida* sp.	8	2-G
	Soybean	8	3-C
$BrToC04_{326}$	Brinjal	7	2-F
	Tomato	7	3-B
$CoToB10_{471}$	Cotton	7	2-C
	Tomato	6	3-A

*Out of 10 whitefly individuals collected from field population of respective plant crop (weed in case of *Sida* sp.) that were PCR amplified using respective SCAR primers

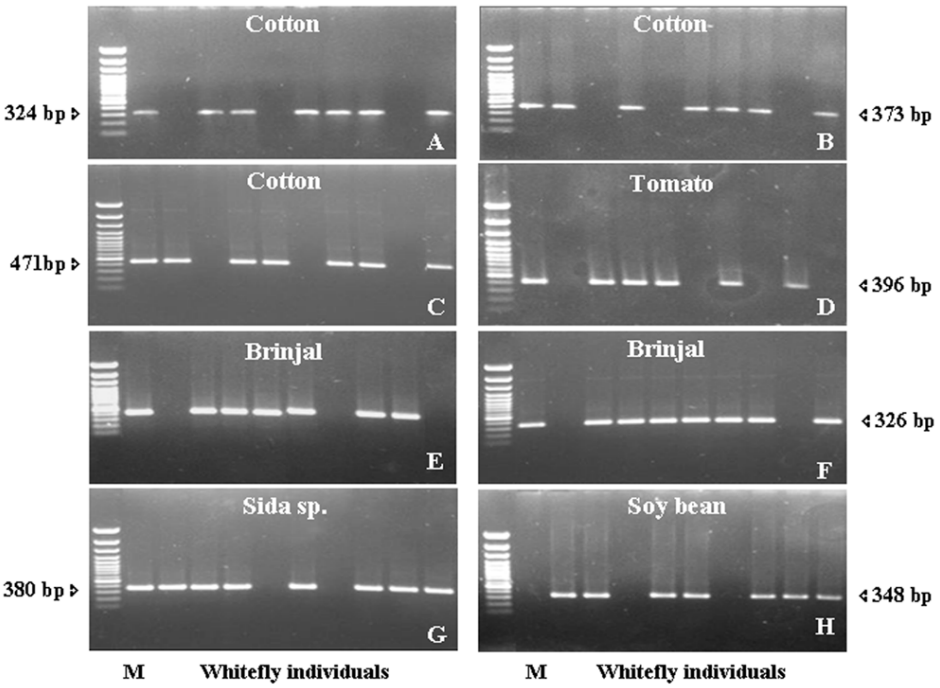

Fig. 2—Detection of plant host specific whitefly individuals by different SCAR markers in whitefly populations collected from fields of respective host plants. Scar Primers: A-CoC03324, B-CoToB10373, C-CoToB10471, D-ToB05396, E-BrC03445, F-BrToC04326, G-SiSoC04380, H-SoH16348, & M- 100 bp DNA size marker lane (MBI Fermentas).

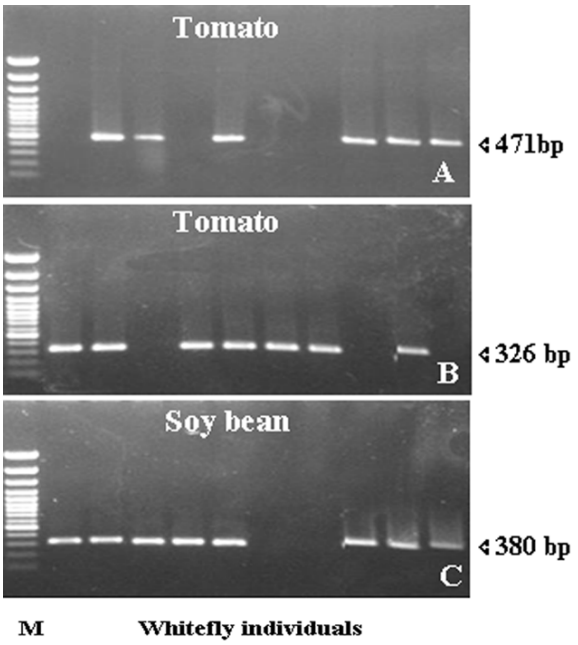

Fig. 3—Detection of whitefly individuals by different SCAR markers with two host specificity in whitefly populations collected from tomato and soybean fields. Scar Primers: A-CoToB10471, B-BrToC04326, C-SiSoC04380, & M- 100 bp DNA size marker lane (MBI Fermentas).

Thus, the specificity of all the 8 SCAR primers was established by amplification of specific SCAR marker of expected size, where most of the individuals in all the populations supported expected amplification. However, some individuals in all the populations failed to be detected by the amplification with the respective SCAR markers. This could be due to mixed population effect, wherein the field population gets mixed with whitefly from alternate source crops and remain undetected. However, the finding that majority of the individuals being detected by the respective markers support the definite existence of host selection of whitefly by the plant host type in nature. Further development of multiplex PCR using more than one type of SCAR markers will simplify the molecular identification of specific whitefly genotypes in a field population.

Where the above findings suggest the existence of host plant selection of whitefly genotypes, identification of whitefly genotypes specific to other plant hosts in a specific geographic regions and development of SCAR markers will find more useful applications in the in-depth and precise analysis of different ecological aspects of *B. tabaci*, particularly in molecular tracking of the movement of this pest amongst different crops weeds and its role as vector of different geminiviral diseases. The elucidation of host specificity in *B. tabaci* populations proliferating on different host crops in the same geographical area will also provide an insight into mechanisms involved in evolution of new races/biotypes in this agronomically important pest species.

References

1 Oliveira M R V, Henneberry T J & Anderson P, History, current status and collaborative research projects for *Bemisia tabaci*, *Crop Prot*, 20 (2001) 709-723.

2 McKenzie C L, Anderson P & Villarreal N, An extensive survey of *Bemisia tabaci* (Homoptera: Aleyrodidae) in agricultural ecosystems in Florida, *Fla Entomol*, 87 (2004) 403-407.

3 Simmons A M, Harrison Jr H F & Ling K, Forty-nine new host plant species for *Bemisia tabaci* (Hemiptera: Aleyrodidae), *Entomol Sci* (Japan), 11 (2008) 385-390.

4 Gawel N J & Bartlett A C, Characterization of differences between whiteflies using RAPD-PCR, *Insect Mol Biol*, 2 (1993) 33-38.

5 Bedford I D, Markham P G, Brown J K & Rosell R C, Geminivirus transmission and biological characterization of whitefly (*Bemisia tabaci*) types from different world regions, *Ann Appl Biol*, 125 (1994) 311-325.

6 Brown J K, Frohlich D R & Rosell R C, The sweetpotato/silverleaf whiteflies: Biotypes of *Bemisia tabaci* (Genn.), or a species complex?, *Annu Rev Entomol*, 40 (1995) 511-534.

7 Rosell R C, Bedford I D, Frohlich D R, Gill R J, Markham P G *et al*, Analyses of morphological variation in distinct populations of *Bemisia tabaci* (Homoptera: Aleyrodidae), *Ann Entomol Soc Am*, 90 (1997) 575-589.

8 Frohlich D R, Torres J I, Bedford I D, Markham P G & Brown J K, A phylogeographical analysis of *Bemisia tabaci* species complex based on mitochondrial DNA markers, *Mol Ecol*, 8 (1999) 1683-1691.

9 Brown J K, Perring T M, Cooper A D, Bedford I D & Markham P G, Genetic analysis of *Bemisia* (Hemiptera: Aleyrodidae) populations by isoelectric focusing electrophoresis, *Biochem Genet*, 38 (2000) 1573-4927.

10 Perring T M, Cooper A D, Rodriguez R J, Farrar C A & Bellows T S J, Identification of a whitefly species by genomic and behavioral studies, *Science*, 259 (1993) 74-77.

11 Boykin L M , Shatters Jr R G, Rosell R C, McKenzie C L, Bagnall R A *et al*, Global relationships of *Bemisia tabaci* (Hemiptera: Aleyrodidae) revealed using Bayesian analysis of mitochondrial COI DNA sequences, *Mol Phylogenet Evol*, 44 (2007) 1306-1319.

12 Singh J, Sohi A S, Mann H S & Kapur S P, Studies on whitefly, *Bemisia tabaci* transmitted cotton leaf curl disease in Punjab, *J Insect Sci*, 7 (1994) 194-198.

13 Berlinger M J, Host plant resistance to *Bemisia tabaci*, *Agric Ecosyst Environ*, 17 (1986) 69-82.

14 Inbar M & Gerling D, Plant-Mediated interactions between whiteflies, herbivores and natural enemies, *Annu Rev Entomol*, 53 (2008) 431-448.

15 Mound A L, Host-correlated variations in *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), *Proc R Entomol Soc Lond Ser A, Gen Entomol*, 38 (1963) 171-180.

16 Mohanty A K & Basu A N, Effect of host plants and seasonal factors on intraspecific variations in pupal morphology of the whitefly vector, *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae), *J Entomol Res*, 10 (1986) 19-26.

17 Palaniswami M S, Nair R R, Pillai K S & Thankappan M, Whiteflies on cassava and its role as vector of cassava mosaic disease in India, *J Root Crops*, 22 (1996) 1-8.

18 Lisha V S, Antony B, Palanaswami M S & Henneberry T J, *Bemisia tabaci* (Homoptera: Aleyrodidae) biotypes in India, *J Econ Entomol*, 96 (2003) 322-327.

19 De Barro P J & Driver F, Use of RAPD-PCR to distinguish the B-biotype from other biotypes of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), *Aust J Entmol,* 36 (1997) 149-152.

20 Da Cunha Galvao L M C, Randomly amplified polymorphic DNA (RAPD), in *Molecular biomethods handbook*, 2nd edn, edited by J M Walker & R Rapley (Humana Press, NJ, USA) 2008, 133-147.

21 Sharma R K, Gupta V K, Jindal J & Dilawari V K, Host associated genetic variations in whitefly, *Bemisia tabaci* (Genn.), *Indian J Biotechnol*, 7 (2008) 366-370.

22 Guirao P, Beitia F & Cenis J L, Biotype determination of Spanish populations of *Bemisia tabaci* (Hemiptera: Aleyrodldae), *Bull Entomol Res*, 87 (1997) 587-593.

23 Moya A, Guirao P, Cifuentes D, Beitia F & Cenis J L, Genetic diversity of Iberian populations of *Bemisia tabaci* (Hemiptera: Aleyrodidae) based on random amplified polymorphic DNA-polymerase chain reaction, *Mol Ecol*, 10 (2001) 891-897.

24 Lima L H C, Navia D, Inglis P W & Oliveira M R V, Survey of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) biotypes in Brazil using RAPD markers, *Genet Mol Biol,* 23 (2000) 1-5.

25 Martinez S S, Carvalho A O R, Vieira L G, Nunes L M & Bianchini A, Identification, geographical distribution and host plants of *Bemisia tabaci* (Genn.) biotypes (Homoptera: Aleyrodidae) in the state of Parana, Brazil, *Ann Soc Entomol Brasil,* 29 (2000) 597-603.

26 Sharma R K, Pal Y, Jindal J, Gupta V K & Dilawari V K, Efficiency of whitefly populations from different hosts for acquisition and transmission of CLCuV, in *Proc 1st Cong Insect Sci* (Punjab Agricultural University, Ludhiana, India), 1 (2005) 67-69.

27 Zheng C, Chang R, Qiu L, Chen P, Wu X *et al*, Identification and characterization of a RAPD/SCAR marker linked to a resistance gene for soybean mosaic virus in soybean, *Euphytica*, 132 (2003) 199-210.

28 Lee G P, Lee C H & Kim C S, Molecular markers derived from RAPD, SCAR and the conserved 18S rDNA sequences for classification and identification in *Pyrus pyrifolia* and *P. communis, Biomed Life Sci*, 108 (2004) 1487-1491.

29 Warude D, Chawan P, Joshi K & Patwardhan B, Development and application of RAPD-SCAR marker for identification of *Phyllanthus emblica* Linn., *Biol Pharm Bull*, 29 (2006) 2313-2316.

30 Rugienius R, Sikesnianas T, Stanys V, Gelvonaukiene & Bendokas V, Use of RAPD and SCAR markers for identification of strawberry genotypes carrying red stele (*Phytophthora fragriae*) resistance gene RPf1, *Agron Res*, 4 (2006) 335-339.

31 Ko C-C, Hung Y-C & Wang C-H, Sequence characterized amplified region markers for identifying biotypes of *Bemisia tabaci* (Hem., Aleyrodidae), *J Appl Entomol*, 31 (2007) 542-547.

32 Gupta V K, Sharma P, Sharma R, Jindal J & Dilawari V K, Development of SCAR markers for specific identification of B-biotype of *Bemisia tabaci* (Gennadius), *Ann Plant Prot Sci*, 1 (2008) 274-281.