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Molecular characterization of whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) populations infesting cassava

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Abstract

Bemisia tabaci (Gennadius) populations, collected from cassava and other plants in major cassava-cultivation areas of Sub-saharan Africa and from elsewhere around the world, were studied to determine their biotype status and genetic variation. Random amplified polymorphic DNA–polymerase chain reaction (RAPD–PCR) markers were used to examine the genetic structure of the populations. The dendogram obtained using the neighbour joining method (NJ) split the cassava-associated populations from the non-cassava types with a 100% bootstrap probability. Analysis of molecular variance (AMOVA) of the RAPD fragments revealed that 63.2% of the total variation was attributable to differences among populations, while the differences among groups (host) and within populations accounted for 27.1 and 9.8% respectively. Analysis of the internally transcribed spacer region I (ITS 1) of the ribosomal DNA confirmed that the cassava populations of *B. tabaci* populations were distinct from non-cassava populations. Experiments to establish whitefly populations on various host plants revealed that cassava-associated populations were restricted to cassava only, whereas *B. tabaci* from other hosts were polyphagous but did not colonize cassava. Hence, populations of *B. tabaci* from cassava in Africa represent a distinct group.

Introduction

Cassava *Manihot esculenta* Crantz (Euphorbiaceae) is the third largest source of carbohydrates for man in the world and is one of the most important crops in Africa (Fauquet & Fargette, 1990). Pests and diseases present a serious impediment to cassava production, with the African cassava mosaic virus (ACMV), an archetypal African virus (Harrison *et al.*, 1986) being the major biotic constraint (Fauquet & Fargette, 1990; Geddes, 1990).

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The only known insect vector of ACMV is the whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (Mound & Halsey, 1978). The virus and its insect vector are found wherever cassava is grown in Africa (Fauquet & Fargette, 1990). *Bemisia tabaci* has been generally considered as a pest of secondary importance (Bird, 1957; Bird & Maramorosch, 1975; Costa, 1976) but, since the early 1980s, this insect has become important due to its high levels of plant infestation, virus transmission and increased resistance to insecticides (Dittrich *et al.*, 1985; Perry, 1985; Gonzales *et al.*, 1992; Cahill *et al.*, 1994). The often indiscriminate use of insecticides, allowing the selection pressure for resistance development in insects (Cock, 1993), and changes in agronomic practices favouring monoculture (Brown *et al.*,

1995), have been considered as being largely responsible for the emergence of *B. tabaci* as a primary pest in tropical and sub-tropical agricultural systems (Brown *et al.*, 1995).

It is generally accepted that morphologically indistinguishable populations with different biological traits (biotypes) exist within the *B. tabaci* species complex (Bird, 1957; Costa & Russell, 1975; Bird & Maramorosch, 1978). Their genetic variability has been studied by using different biochemical and molecular approaches. The electrophoretic profiles of esterases, malate dehydrogenases and α-glycerophosphate dehydrogenases have been investigated (Wool *et al.*, 1989; Costa *et al.*, 1993; Byrne *et al.*, 1995) and provide evidence of polymorphism in *B. tabaci* populations. Bedford *et al*. (1992) used these markers to characterize biotypes J, E and L collected on *Ipomoea congesta* Brown (Convolvulaceae) in Nigeria, *Asystacia gangetica* L. (Acanthaceae) in Benin and *Gossypium hirsutum* L. (Malvaceae) in Sudan, respectively. The B-biotype, which was first identified in the USA (Costa & Brown, 1991; Cohen *et al.*, 1992), has received greater attention because of its unique biochemical and biological characteristics (Costa *et al.*, 1991; Bellows *et al.*, 1994). Previous studies have proved the usefulness of amplified fragment length polymorphism (AFLP) (Cervera *et al.*, 2000) and random amplified polymorphic DNA–polymerase chain reaction (RAPD–PCR) techniques in distinguishing biotypes, host affiliation and geographical races of *B. tabaci* (Gawel & Bartlett, 1993; Perring *et al.*, 1993; Bedford *et al.*, 1996; De Barro & Driver, 1997; Moya *et. al.*, 2001). Although RAPD analysis is not suitable for defining the taxonomic status of insects (Gawel & Bartlett, 1993), it is quite useful in distinguishing closely related populations (Banks *et al.*, 1998; Guirao *et al.*, 1997).

DNA sequence data from mitochondrial and ribosomal genes are increasingly being used to estimate phylogenetic relationships both within *B. tabaci* and between this species and other whitefly species (Clark *et al.*, 1992; Campbell, 1993; Frohlich *et al.*, 1999; De Barro *et al.*, 2000). In general, rRNA genes are highly conserved, but the non-translated regions flanking those genes evolve at a much higher rate and are highly variable (Hillis & Dixon, 1991). This makes them useful for constructing phylogenies of closely related species and populations. Recently, the internally transcribed spacer (ITS) sequence analysis has gained popularity for the study of within-population differences. Much of this is due to the availability of universal primers for PCR amplification (White *et al.*, 1990), and the lack of internal repetitive structures that make ITS accessible to direct sequence analysis. In addition, the evolution pattern of ITS is not as complex as it is found in the intergenic spacer region (IGS) (Hillis & Dixon, 1991; Schlötterer, 1998).

Bemisia tabaci is primarily known as a polyphagous insect species (Mound, 1983) and thus, host affiliations are not considered to be important in biotype denominations. For cassava, however, a host specialization of *B. tabaci* was observed (Burban *et al.*, 1992) among West African populations, but this was not studied using molecular markers that had been used to characterize other world populations. Furthermore, cassava-growing regions of Africa are very diverse and it is unlikely that cassava affiliated *B. tabaci* populations are uniform in biological or genotypic characters. This study was initiated to study whitefly populations found on cassava, to reveal the genetic relationships among *B. tabaci* populations associated with cassava in Africa and to determine their biotype affiliations.

Materials and methods

Insect populations

Adult *B. tabaci* were collected from cassava in major growing areas of Africa and preserved in 70% alcohol until use. Sampling was done on isolated farms where only cassava was planted. Samples from other hosts within Africa were also collected. Outside Africa, samples from both cassava and non-cassava were included in the study as indicated in table 1. Larval stages of the insects were also collected for morphological identification following the guidelines described by Martin *et al*. (2000). Where possible, live cultures were established in the laboratory. Reference samples of *B. tabaci* biotypes A and B were kindly provided by J.K. Brown (Department of Plant Sciences, University of Arizona). These two biotypes have been characterized with respect to mitochondrial genes (Campbell, 1993; Frohlich *et al.*, 1999) and esterases (Brown *et al.*, 2000). Samples of biotype Q were provided by D. Jansen (Centro de Investigacion y Formacion Horticola (CIFA), Almeria, Spain), and biotype S by I. Bedford (John Innes Centre (JIC), Norwich, UK). *Bemisia afer* Priesner & Hosni (Hemiptera: Aleyrodidae) was donated by J. Colvin (Natural Research Institute (NRI), Chatham, UK) and used in this study as an out-group.

To determine alternative hosts, *B. tabaci* populations reared on cassava were introduced to different plants (tomato, cowpea, sweet potato, okra, cotton, lima bean and *Nicotiana tabacum* L. (Solanaceae)) and kept under prevailing environmental conditions. Populations from other hosts were introduced to cassava in the same manner. In both cases, the insects did not survive.

DNA extraction

The insects were sexed before extracting their DNA. Single insects were transferred into sterile 1.5 ml reaction tubes and washed with distilled water. Total nucleic acid was extracted using the High Pure PCR Template preparation kit (Roche, Mannheim, Germany), essentially following the manufacturer's recommendations. DNA was measured by fluorimetry (DyNA Quant 200, Hoefer, San Francisco, USA) and a defined concentration used in RAPD–PCR, and for amplification of the ITS rDNA.

RAPD–PCR

Sixty 10-mer oligonucleotide single primers (Operon) were screened for their ability to provide a suitable banding pattern, and 10 (Operon B_8, B_{11}, B_{14}, B_{20}, C_1, C_4, C_8, C_{14}, F_{12} and H_{16}) were selected for this study. The RAPD–PCR reaction was done in a 50 μl volume containing 6 ng template DNA, 5 μl of 10X reaction buffer, 6 mM $MgCL_2$, 0.1 mM dNTP, 0.4 μM primer and 2.5 U *Taq* polymerase (Promega, Mannheim, Germany). Amplification was performed using the following temperature cycle: Initial denaturation for 2.30 min at 94°C, followed by 40 cycles of 92°C for 20 s, 38°C for 15 s, ramp at 0.3°C s^{-1} to 72°C, and 72°C for 1 min, and a final extension of 7 min at 72°C.

RAPD products were separated by electrophoresis on a 2% agarose gel (Seakem, FMC Bioproducts, USA), containing ethidium bromide and using a mixture of 100 bp and 1 kb lamda DNA (Biolabs, Frankfurt, Germany) as

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molecular size marker. Separate analyses of male and female insects were conducted. To avoid the problem of dominance, only males were used for the analysis of population diversity. The banding pattern was evaluated by scoring 1 for a band present and 0 for a band absent. The character data obtained was imported in Arlequin ver 2.00 (AMOVA, Schneider *et. al*., 2000) to investigate the genetic structure of the populations. Matrix of Euclidean squared distances was obtained and used in performing the analysis of molecular variance (AMOVA), partitioning the total variance into: among groups (AG), among populations within group (AP/WG) and within population (WP). The AG variance represents the variation among the host-associated populations (cassava and non-cassava), while the AP/WG variance stands for the differences between the sub-populations within the host-associated populations. Populations were grouped according to the clusters identified. The significance of the variance components was tested with 1000 permutations. The distance matrix was also used in PHYLIP 3.5c (Felsenstein, 1994) to construct a dendogram using the neighbour-joining algorithm (Saitou & Nei, 1987). The stability of the tree topology was determined with 1000 bootstrap tests.

Amplification and cloning of ITS rDNA

To confirm the result of RAPD–PCR, 33 of the 39 *B. tabaci* populations were subjected to ITS analysis. Two universal ITS primers, ITS 1 and ITS 4, derived from conserved regions of the 18S and 28S ribosomal genes of fungi (White *et al*., 1990) were used for initial amplification of the entire ITS 1 and ITS 2 rDNA of *B. tabaci*. Upon retrieval of primary sequence information, the primers Bem IT1U24: 5′-CCG TAG GTG AAC CTG CGG AAG GAT-3 and BemIT4L24: 5′-TTC CTC CGC TTA TTG ATA TGC TTA-3 were designed from the 18S and 28S regions, for specific amplification of whitefly ITS. The ITS is flanked at the 5′ and 3′ end by the small (18S) and the large (28S) rDNA respectively, while the 5.8S is embedded in the ITS region.

Polymerase chain reaction (PCR) was performed in a 50 μl reaction volume using 100 pmol of each primer and 4 ng of template DNA, 25 μl of a premixed 2 X PCR buffer (consisting of 3 mM $MgCl_2$, 100 mM Tris pH 8.3, 100 mM KCl, 400 μM each of dNTPs, and 3 M Betaine) and 2.5 units of *Taq* polymerase (Promega, Mannheim, Germany). The reaction mixture was subjected to the following temperature cycle: an initial denaturation at 95°C for 3 min followed by 30 cycles of 95°C, 30 s, 50°C, 1 min and 72°C, 1 min, and a final extension of 10 min at 72°C terminating amplification. Control reactions without DNA were included in each PCR.

For cloning, PCR products were separated by electrophoresis on 1% agarose gels, excised, and purified using the QIAEX II agarose gel extraction protocol (QIAGEN GmbH, Hilden, Germany). Purified dsDNA fragments were ligated into a pGEM-T vector (Promega, Mannheim, Germany) and subsequently transferred into *Escherichia coli* JM 109 cells. Nucleotide sequences of the cloned PCR products were determined by cycle sequencing using a fluorescent dye dideoxy chain termination sequencing reaction kit and an applied Biosystems 373 A sequencer (PE – Applied Biosystems). Sequencing reactions were primed on both strands using either the T7 or Sp6 promoter sequences of the pGEM-T vector and/or internal annealing sites with specific primers. For DNA sequencing, the services of a commercial company were used (Sequiserve, Vaterstetten, Germany).

Data analysis and reconstruction of a phylogenetic tree

For sequence comparison, *Bemisia* ITS 1 sequences comprising approximately 550 nucleotides were analysed. There were only negligible variations in amplicon length, with little inconsistencies due to variable length C-repeats. ITS 1 sequences were submitted to the European Molecular Biology Laboratory (EMBL) database with accession numbers indicated in table 1. Nucleotide sequences obtained in this study were compared with rDNA ITS 1 sequences of several *B. tabaci* species retrieved from the Genbank/EMBL database (see fig. 3).

Sequence alignment was done using the Clustal W (Thompson *et al*., 1994) alignment routine. Clustal alignments were used for inferring phylogenetic trees using the neighbour joining-method developed by Saitou & Nei (1987). To determine the confidence values for the grouping within a tree, a bootstrap analysis (Felsenstein, 1985) was performed using 1000 resamplings of the data. Internally transcribed spacer 1 of *B. afer* was used as an out-group in clustal analysis.

Results

RAPD–PCR

The populations of *B. tabaci* obtained from different locations and host plants in Africa and elsewhere, that were analysed by RAPD–PCR are shown in table 1. After a series of repeated trials, reproducible bands common to cassava-associated populations were identified. Using the primer H_{16}, three prominent dsDNA fragments of about 700, 850 and 1040 bp in size, and common to all *B. tabaci* from cassava in Africa, were observed. The RAPD fragments obtained from PCR amplification of cassava whitefly specimens from India were different with four bands of approximately 750, 850, 950, and 1100 bp in size (fig. 1, lanes 5 and 6).

The matrix of the differences among the 39 populations was translated into a tree diagram using the NJ method (fig. 2). The first major split on the dendogram separated the cassava-associated populations from the non-cassava types, with all cassava-type populations forming a cluster in 100% of the bootstrap tests. Populations from East Africa clustered together and included all populations from the Democratic Republic of the Congo (DRC, former Zaire). The Indian cassava-populations formed a separate cluster, branching out at a considerable length from the African group. The stability of this branch (98%) gives a strong indication that these populations are different.

On the other branch of the tree, the non-cassava African populations and the old world biotypes bifurcate and this was supported in 71% of the bootstrap tests. All populations collected from cowpea, cotton and tomato in Africa and the population from the UK collected on tomato constitute one group of the bifurcate that includes the silverleafing biotype. The other non-cassava populations, including the tomato population from Iran, formed the other group. The separation of the populations from the Indian sub-continent from the rest of non-cassava populations was supported in 87% of the stability tests.

In AMOVA (host plant, region and locality of the populations defined hierarchically in that order as the source

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Table 1. *Bemisia tabaci* populations used in RAPD–PCR and ITS analysis for biotype identification.

Species/ biotype	Location	Acronym	Host	GenBank acc. no.
Bemisia tabaci	Ibadan (Nigeria)	Nigeria-Cowpea38	Cowpea	AJ315797
Biotype Q	Almeria (Spain)	Spain-Cucumber18	Cucumber	AJ315795
B. tabaci	Agadir (Morocco)	Morocco-Cucumber48	Cucumber	AJ315802
Biotype B	Arizona (USA)	USA-Cotton19	Cotton	AJ315821
Biotype A	Arizona (USA)	USA-Cotton20	Cotton	AJ315796
B. tabaci	Jalna (India)	India-Cotton47	Cotton	AJ315804
Biotype S	Norwich (UK)	Spain-*Ipomoea*59	*Ipomoea* spp.	AJ315820
B. tabaci	Jalna (India)	India-Eggplant44	Eggplant	AJ315803
B. tabaci	Bovar (CAR)	CAR-Cassava24	Cassava	AJ315815
B. tabaci	Kisangani (Congo)	DRC-Cassava55	Cassava	AJ315809
B. tabaci	Kampala (Uganda)	Uganda-Cassava50	Cassava	AJ315811
B. tabaci	Bussia (Kenya)	Kenya-Cassava60	Cassava	AJ315808
B. tabaci	Abidjan (Cote d'lvoire)	CI-Cassava28	Cassava	AJ315814
B. tabaci	Nzerekore (Guinea)	Guinea-Cassava30	Cassava	AJ315813
B. tabaci	Bouake (Cote d'lvoire)	CI-Cassava26	Cassava	AJ313805
B. tabaci	Karaj (Iran)	Iran-Tomato56	Tomato	AJ315799
B. tabaci	Norwich (UK)	UK-Tomato49	Tomato	AJ315801
B. tabaci	Kisangani (DR Congo)	DRC-Tomato52	Tomato	AJ315800
B. tabaci	Ada (Ghana)	Ghana-Cassava33	Cassava	AJ315812
B. tabaci	Ibadan (Nigeria)	Nigeria-Cassava05	Cassava	AJ315819
B. tabaci	Onne (Nigeria)	Nigeria-Cassava06	Cassava	AJ315818
B. tabaci	Bangalore (India)	India-Cassava65	Cassava	AJ315807
B. tabaci	Trivandrum (India)	India-Cassava66	Cassava	AJ315806
B. tabaci	Limbe (Cameroon)	Cam-Cassava23	Cassava	na
B. tabaci	Lusaka (Zambia)	Zambia-Cassava07	Cassava	AJ315817
B. tabaci	Gbojome (Togo)	Togo-Cassava31	Cassava	na
B. tabaci	Kinshasha (DR Congo)	DRC-Cassava17	Cassava	AJ315816
B. tabaci	Kisangani (DR Congo)	DRC-Cassava53	Cassava	AJ315810
B. tabaci	Kaiama (Nigeria)	Nigeria-Tomato35	Tomato	na
B. tabaci	Aboki (Nigeria)	Nigeria-S.potato36	Sweet potato	na
B. tabaci	Katsina (Nigeria)	Nigeria-Tomato37	Tomato	na
B. afer	Kent (UK)	Baf57UK	Tomato	AJ315822
B. tabaci	Coimbatore (India)	India-Eggplant43	Eggplant	na
B. tabaci	Abidjan (Cote d'lvoire)	CI-Cowpea29	Cowpea	AJ509018
B. tabaci	Ibadan (Nigeria)	Nigeria-Limabean78	Limabean	AJ509017
B. tabaci	Fontem (Cameroon)	Cam-Pepper81	Pepper	AJ509021
B. tabaci	Cotonou (Benin)	Benin-Soybean80	Soybean	AJ509019
B. tabaci	Atakpane (Togo)	Togo-Potato79	Potato	AJ509016
B. tabaci	Funtua (Nigeria)	Nigeria-Cotton77	Cotton	AJ509021

CAR, Central African Republic; DRC, Democratic Republic of the Congo; CI, Cote d'Ivoire; Cam, Cameroon; na, samples not included in the sequence analysis.

of variation), 63.2% of the total variance was attributed to differences between populations within the host group (table 2). The differences between the cassava- and non-cassava-associated populations contributed only 27.1% to the total variance, while within population differences accounted for 9.8%. The fixation index (table 2) serves as a convenient measure of genetic differences between populations. Observed values of F_{ST} in natural populations include not only random drift, but also migration, natural selection, and mutation. From this analysis, it is evident that the observed genetic variations between cassava- and non-cassava type populations could not have occurred by chance. Although the within population differences accounted for a limited amount of the total variance, significant diversity exists within them.

Analysis of the internally transcribed spacer regions of ribosomal DNA

Using specific primers (Bem IT1U24 and Bem IT4L24) flanking ITS 1 and ITS 2 region, PCR products of about 1200 to 1400 bp were obtained. However, sequencing reactions resulted in abrupt stops at approximately 190 nt upstream of the 28S rDNA start site and hence contigs (a composition of over-lapping sequences forming one continuous sequence) were only reached for a few samples. Consequently, the nucleotide analysis was based on the ITS 1 sequence comparison (approximately 550 bp) with ITS 1 sequences from world populations of *B. tabaci* (De Barro *et al.*, 2000) included in the alignment.

A total of 106 informative sites were obtained from the

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Table 2. Hierarchical analysis of molecular variance on matrix of distances between haplotypes of *Bemisia tabaci* from cassava and non-cassava hosts.

Source of Variation	d.f	Sum of squares	Variance component	Variation (%)
Between host plant (AG)	1	1775.7	9.47 Va	27.1
Between populations (regions) within host plant (AP/WG)	7	4103.8	22.1 Vb	63.2
Within populations in locality (WP)	235	803.6	3.42 Vc	9.78
Total	243	6683.2	35.0	

Fixation indices: F_{SC}, 0.86590; F_{ST}, 0.90220; F_{CT}, 0.27072.

Va, variance component due to differences between host plant (group); Vb, variance component due to differences between populations within host plant; Vc, Variance component due to differences within population in the same locality.

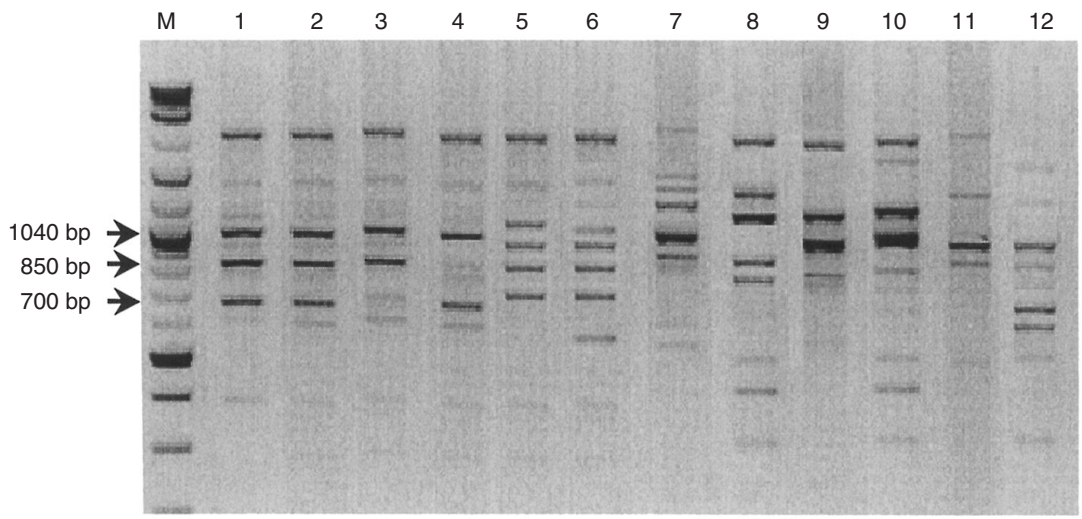

Fig. 1. Random amplified polymorphic DNA-polymerase chain reaction (RAPD–PCR) fragments generated from *Bemisia tabaci* obtained from different host plants and locations, using the Operon primer H_{16}. Lanes 1–3, cassava Africa; lane 4, biotype S; lanes 5 and 6, cassava India; lane 7, eggplant India; lane 8, cowpea Nigeria; lane 9, biotype Q; lane 10, biotype B; lane 11, biotype A; lane 12, *B. afer*. M, 100 bp/kb molecular weight marker mix (BIOLABS, UK).

alignment. Areas of sequence divergence involved nucleotide deletions, insertions and substitutions. About 60% of the nucleotide substitutions were transversions. Almost all cases of indels (nucleotide insertions and deletions) observed were between outgroup and ingroup populations. The only case of deletion within the *B. tabaci* populations occurred at position 476, and it involved non-cassava populations.

Comparison of ITS 1 rDNA sequences of Bemisia tabaci

The alignment of ITS 1 rDNA sequences revealed that *B. tabaci* populations were highly similar, with genetic distances ranging between 0 and 0.089. All *B. tabaci* specimens fell into two distinct clusters segregating the cassava-associated populations from populations obtained from other hosts (fig. 3). This host separation was the same even when all the available 126 ITS 1 sequences were included in the analysis. The only exception to this distinct separation was presented by sequences of one population from Benin found on *Asystasia* aligning with ITS 1 sequences from cassava populations. However, the original host is contentious. Within the cassava whitefly cluster, all sequences were more than 99% identical. Cassava-associated *B. tabaci* populations showed a closer relationship to biotype A, with 94–95% identical nucleotides, than to biotypes B (91.3–93.2%) and Q (92.6–93%). This demonstrated that cassava-associated populations of *B. tabaci* were not affiliated to any of the established biotypes. Only the sequences of the *B. tabaci* biotype S (this analysis and De Barro *et al.*, 2000) clustered with the cassava populations (fig. 3), revealing a very high nucleotide sequence identity (97.1–98.3%).

Bemisia tabaci populations obtained from plants other than cassava formed different clusters. Unlike in RAPD analysis, biotypes B and Q fell into different branches of a bifurcate, and the cucumber population from Morocco occurred at the root to these two clades (fig. 3). However, the separation of these clades was not supported by a high bootstrap probability. *Bemisia tabaci* populations from India clustered with populations from Pakistan, Taiwan, Nepal and Turkey and this clade showed a closer relationship to biotype A (96–97.1%) than to biotype B (95.4–96%).

A sample of *Bemisia* collected from cassava in Zambia was related to *B. afer*, sharing > 95% nucleotide identity. The genetic distance of this population with its host allies ranged between 0.26 and 0.28; hence it was assumed to be *B. afer*.

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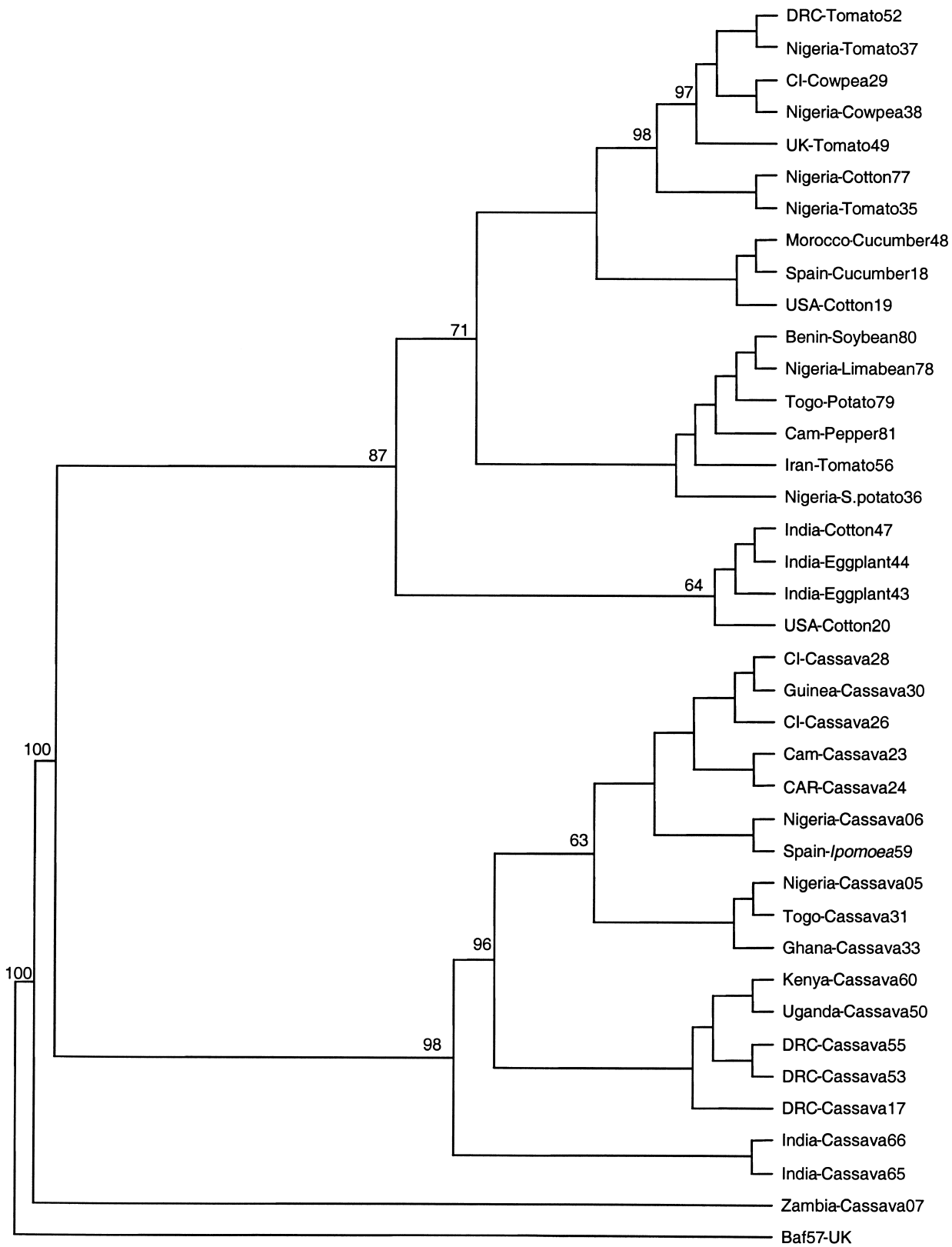

Fig. 2. Dendrogram deduced from matrix of pairwise distances in RAPD–PCR analysis between 39 populations of *Bemisia tabaci*, using the neighbour-joining method. Numbers on tree reflect bootstrap values (%). Samples and acronyms are indicated in table 1.

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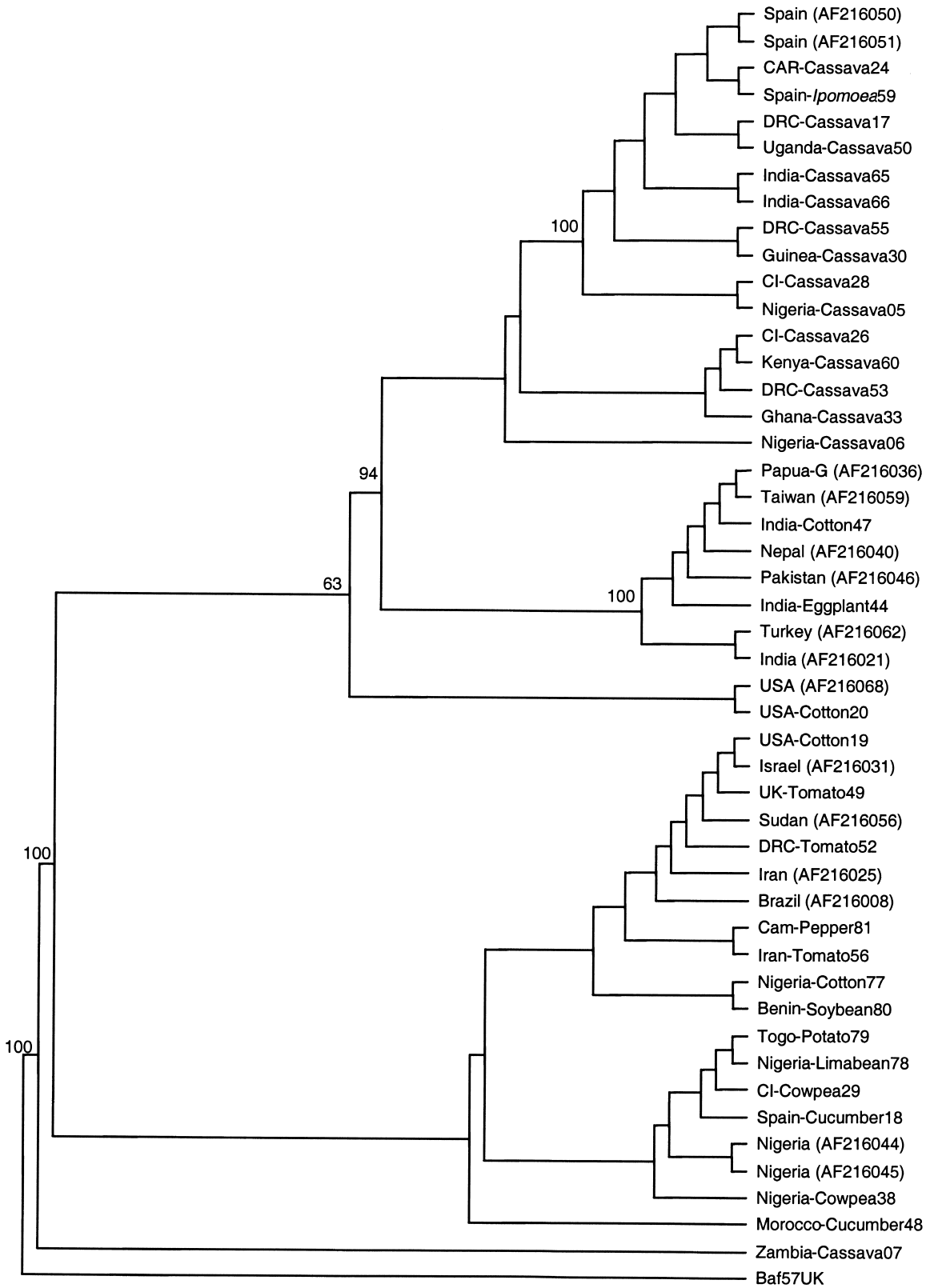

Fig. 3. Phylogenetic tree constructed from ITS 1 nucleotide sequences from *Bemisia tabaci* and other whiteflies collected from different host plants and geographical regions. Numbers on tree reflect bootstrap values (%). Samples represented by acronyms are indicated in table 1. Sequences with accession numbers were obtained from Genebank/EMBL (De Barro *et al.,* 2000).

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Comparison of ITS 1 sequence and RAPD-PCR analysis

Both techniques revealed similar results when larger branches of the tree for RAPD and the ITS 1 sequence alignment were compared. Cassava-associated *B. tabaci* populations were assigned by both methods of analysis to a cluster, separate from all other populations of *B. tabaci*. The genetic distances within the cassava-associated populations and between cassava and non-cassava clusters were equally large. The resolution capacity of RAPD for studying whitefly species, however, proved more stringent than ITS 1 analysis. This is emphasized by a deeper reflection of the differences among close neighbours and relatives. Whereas with ITS 1 analysis, a geographical discrimination of cassava *B. tabaci* was not resolved, RAPD analysis made a geographical origin of the whitefly populations visible by separating an East African from a West African branch.

Discussion

This comparative study has demonstrated that *B. tabaci* populations infesting cassava are distinct from *B. tabaci* found on other hosts, a finding that concurs with other experiments involving different markers (Burban *et al.*, 1992; Legg *et al.*, 2002). Molecular tools were used to elucidate the biotype affiliations of cassava-associated *B. tabaci* populations and to determine their genetic differences with other populations. Sequence analysis of the ITS 1 was used for identification of host-associated populations while subsequent RAPD analysis further resolved genetic variations among *B. tabaci* populations from different regions of the world. Great effort was made to ensure reproducibility of RAPD analysis and differentiation of *B. tabaci* populations was therefore not based on the evaluation of unique RAPD fragments being characteristic of a particular population, but on polymorphic patterns obtained with a number of separate RAPD approaches. Thus RAPD analysis provided useful information for differentiation of *B. tabaci* populations.

The nucleotide sequence identities of up to 99% among the cassava populations in the ITS 1 region provided evidence that this marker was not useful for resolving differences among cassava-associated populations occurring in different geographical locations. Recently, ITS 1 analysis of *B. tabaci* world populations from diverse regions (De Barro *et al.*, 2000) revealed a biogeographical pattern of *B. tabaci* biotypes. The work of De Barro *et al*. (2000) provided evidence in support of two groups of *B. tabaci*, comprising the non-silverleafing and the silverleafing populations. Several monophyletic clades could be seen within their non-silverleafing group; including two separate clades from the Indian sub-continent, one clade from Australia and two clades from Africa. The result of this current study that aimed to determine the genetic diversity and biotype affiliation, especially of African cassava populations of *B. tabaci*, agrees with this grouping. The non-cassava African populations, while not causing silverleafing, have a close relationship with the silverleafing B biotype. Silverleafing is a physiological disorder induced only by B biotype in squash (Bharathan *et al.*, 1990; Yokomi *et al.*, 1990), and has since being used as a diagnostic marker. De Barro *et al*. (2000) considered the silverleafing symptom a recently evolved character state and that absence of this symptom is a plesiomorphy of the silverleafing clade. The second African clade in the non-silverleafing group presumably represents cassava-associated populations. It is, however, confusing to note that the Benin population was collected from a non-cassava host, *Asystasia*. Nevertheless, the fact that cassava type *B. tabaci* is a host specialist does not exclude it from being found on other plants as a visitor. Certainly, cassava populations do not colonize this weed host.

A striking similarity between the cassava populations and the biotype S reported from Spain was found (Banks *et al.*, 1999, fig. 3 AF216050, AF216051, Spain-*Ipomoea*), confirming the report of Cervera *et al*. (2000) who used AFLP markers to study biotype populations of *B. tabaci*. However, since this biotype was found only once in one location in Spain, and now exists only as a laboratory colony, it was tempting to consider this population as an accidental introduction into Europe and of probable African origin. In host transfer experiments, the cassava-associated populations from West Africa failed to colonize *Ipomoea indica* Burm (Convolvulaceae), the weed host from which biotype S was collected. This supports the view that the biotype S was probably in transit at the time it was collected.

In agreement with the ITS 1 analysis, RAPD results showed that the cassava-associated populations were clearly distinct from other host-associated populations. A further distinction between *B. tabaci* from East Africa and those from West Africa was obvious within the cassava populations. Less than 72% genetic identity of cassava whitefly populations from West and Central Africa was found with the East African group. Pending biological studies to confirm the analysis of molecular variation, any interpretation concerning biotype affiliations or demarcations remain purely speculative. However, the recent report of Legg *et al*. (2002) has shown the existence of two genotype clusters (Ug1 and Ug2) of *B. tabaci* in Uganda, in which one (Ug2) was associated with the cassava mosaic epidemics in that region. Their conclusion was based on the distribution of Ug2 in relation to the occurrence of the epidemics. The idea of a specific *B. tabaci* biotype being responsible for this epidemic, however, seems not to be favoured by the findings of Maruthi *et al*. (2002) in which *B. tabaci* populations from both epidemic and non-epidemic areas transmitted all the African cassava mosaic geminiviruses with equal efficiencies.

It was interesting to note that the analysis of a few cassava-associated *B. tabaci* populations collected from India revealed a distant relationship with their African counterpart. Although these samples might be too few to justify a strong statement, there are indications from previous work (De Barro *et al*., 2000) that the Asian cassava-associated populations are probably polyphagous. The two clades of non-silverleafing populations in India do not appear to be host affiliated (P.J. De Barro, personal communication).

Significant relationships with known *B. tabaci* biotypes were found only with non-cassava-associated *B. tabaci*. Thus the cowpea population used in this study, showed a very close relationship with biotype B (98.8%) and formed a clade with biotype Q from Spain and with a population from Morocco designated as biotype Q. This cowpea population from Nigeria together with populations from Egypt and Sudan were also shown to fall within the major cluster comprising the *B. tabaci* silverleafing biotype (De Barro *et al.*, 2000). The combined results of the two markers used in this

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study have provided evidence that the cassava-associated populations of *B. tabaci* are more distantly related to all the characterized biotypes than the non-cassava African group, while the Indian cassava populations represent a different genotype. Even within the African cassava group, evidence on the occurrence of sub-populations is provided, which might reflect different phenotypes. A broad study of such important species as *B. tabaci* at global level is incapable of resolving genetic differences at micro geographical scale (De Barro *et al.*, 2000). This present study has improved our knowledge of the population structure of the so-called African clade.

The host specialization of *B. tabaci* on cassava in Africa (Dubern, 1979; Abdullahi *et al.*, 1998) could be described as 'host-shifting'. Both cassava and non-cassava *B. tabaci* populations are sympatric in their distribution and host alternation is not necessary for cassava-associated populations since this crop is grown throughout the year and hence can serve as a permanent host. This might have triggered the rapid adaptation of *B. tabaci* to cassava with the subsequent loss of the capacity to feed and propagate on alternative hosts. Since this host adaptation is a fairly recent process with cassava introduction into Africa only in the 15th century (Carter *et al.*, 1995), the diversification of *B. tabaci* on cassava is also at an early stage. However, the analysis of molecular variance indicates that *B. tabaci* populations infesting cassava in Africa are distinct and most likely comprise several biotypes.

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