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Identification of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) based on RAPD and design of two SCAR markers

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A survey was made to assess the biotype status of whitefly *Bemisia tabaci* populations in the agricultural region of north-east of Morocco. The study involved application of the randomly amplified polymorphic DNA technique (RAPD) for three populations of *B. tabaci* collected from different host plants. Results obtained were consistent and showed that the northeastern Moroccan populations of *B. tabaci* were composed of two genetic types, namely the B and Q biotypes. This is one of the first reports about the invasion of the B biotype into Morocco. In this manuscript, we report the development of simple and cost-effective markers that distinguish between the two *B. tabaci* biotypes B and Q, by using locus-specific sequence characterised amplified region (SCAR) markers. Two putative, biotype-specific RAPD markers were generated using the OPA-10 and OPT-01 primers. The SCAR markers were developed from two obtained sequences. Two pairs of primers were designed, and then used to amplify efficiently specific markers for the B and Q biotypes.

Key words: *Bemisia tabaci*, biotypes B and Q, RAPD, SCAR markers.

INTRODUCTION

The aleyrodid insect *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) has emerged in the last decade as a major pest problem, mainly due to its prominent role as a vector of many viral diseases. This whitefly has become a serious constraint to agricultural production in tropical, subtropical and temperate zones (Brown, 1994). One of the regions severely affected by viruses transmitted by *B. tabaci* (particularly geminiviruses) is the Mediterranean Basin. From the mid 1980s, serious epidemics of tomato yellow leaf curl virus (TYLCV) have been reported in Israel, Egypt, Spain, Italy and Morocco (Jones, 2003).

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Biotypes of *B. tabaci* are morphologically identical but differ in biochemical, physiological, and life-history traits, host plant specificity, and virus transmission capability (Costa & Brown., 1991; Wool *et al*., 1993). Furthermore, barriers or limitations to interbreeding among biotypes of *B. tabaci* have been reported (Bedford *et al*., 1994; De Barro & Hart, 2000). Differential susceptibility or resistance to insecticides has also been suggested as contributing to or arising from the biological distinctiveness of biotypes (Costa *et al*., 1993). In southern Europe and Middle East, the two most widespread biotypes are B and Q (Guirao *et al*., 1997; Rosell *et al*., 1997). The B biotype has a broad geographical distribution and is considered to be a recent invader over much of its range (De Barro *et al*., 2005).

In order to distinguish between whitefly biotypes, several biochemical and molecular markers have

been developed. Multiple arbitrary amplicon profiling (MAAP) techniques, such as random amplification of polymorphic DNA (RAPD-PCR), are commonly used in defining *B. tabaci* biotypes (Guirao *et al*., 1997; Horowitz *et al*., 2003). The method enabled simultaneous analysis of a large number of samples, but the obtained banding patterns are often difficult to interpret and at times irreproducible (Guirao *et al*., 1997). Amplified fragment length polymorphism (AFLP) is another PCR-based technology used for the genetic study of *B. tabaci* populations. However, this technique is relatively complex, time-consuming, labour-intensive, and expensive (Vos *et al*., 1995; Cervera *et al*., 2000).

Specific PCR primers also have been designed following sequence analysis of RAPD or AFLP fragments. Such primers amplify single loci and are therefore locus-specific (Ohmori *et al*., 1996; Agusti *et al*., 2000). The amplification of such specific PCR markers is usually referred to as sequence characterised amplified regions (SCAR) (Paran & Michelmore, 1993).

Specific PCR-based polymorphism can be detected by molecular phylogenetic studies based on comparative sequence analysis of mitochondrial COI and 16S rDNA or nuclear DNA (ITS, 18S rDNA). Such analyses have been performed to determine the genetic relationships among *B. tabaci* populations (Campbell *et al*., 1993; De Barro *et al*., 2000). These techniques required the sequencing of PCR products and were relatively expensive and at times not practical for large scale analysis of *B. tabaci* population dynamics (Calvert *et al*., 2001).

In 1997, a severe outbreak of TYLCV (Peterschmitt *et al*., 1999) was recorded for the first time in Morocco. The viral epidemic was associated with a raise of its vector population *B. tabaci* (Jebbour & Abaha, 2002). Little is known about the genetic variability of the *B. tabaci* in Morocco and the knowledge of the genetic variation within whitefly populations is necessary for their efficient control and management. In the work reported herein, we have used RAPD markers to differentiate between *B. tabaci* biotypes. Because of the difficulty in reproducibility, these RAPD markers were then converted into SCAR. This method has the advantages of being simple, cost-effective, rapid, reproducible and applicable to a large number of samples (Paran & Michelmore, 1993).

MATERIALS AND METHODS

Insect populations

Seventy adult whiteflies were collected during 2004, 2005 and 2006 (between May and July) from host plants at several field-sites in north-east of Morocco (Table 1) using a hand-held aspirator. The flies were preserved immediately in absolute ethanol and then stored in a refrigerator at 4°C. All strains were identified as *B. tabaci*, based on the morphology of the pupal and adult stages. Reference samples of *B. tabaci* biotypes B and Q were obtained from the Valencia University (Spain).

DNA extraction

Although the basic strategy of DNA extraction was similar to that reported by Lima *et al*. (2000, 2002), some modifications were performed. Once their sexes were determined, individual female whitefly adults were transferred into sterile 1.5 ml reaction tubes and crushed with a pipette tip in 50 µl of extraction buffer (10 mM Tris-HCl, pH 8, 1 mM EDTA, 0.3% Triton X-100, 60 µg ml^{-1} proteinase K). The homogenate was then incubated at 65°C for 3 h. Samples were boiled for 12 min to inactivate the proteinase K and then stored at –20°C. The DNA was quantified by fluorimetry (Fluorescent DNA Quantitation kit, Bio-Rad).

DNA samples were extracted from 20 individual insect males from each host plant and from 45, 40, 45 and 30 individual insect females from tomato, lan-

TABLE 1. List of *Bemisia tabaci* populations used in this study

Host plant	Source	Biotype	Number of individuals	
			Males	Females
tomato (*Lycopersicon esculentum*)	Berkane		20	45
lantana (*Lantana camara*)	Berkane		20	40
melon (*Cucumis melo*)	Berkane		20	45
tomato	laboratory (Spain)	B	20	30
tomato	laboratory (Spain)	Q	20	30
Total			290	

tana, melon and reference samples, respectively (Table 1) (damaged insects were automatically excluded). It is worth noting that the analyses of females and males were carried out separately.

RAPD analysis

PCR conditions were optimised prior to any amplification. The reaction mixtures were performed in a final volume of 25 µl containing a concentration of 10-15 ng of template DNA, 1× colourless GoTaqTM buffer [10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 1.25 U of the GoTaqTM DNA polymerase (Promega), 200 mM of each dNTP, 2.5 mM $MgC1_2$, 18 pmol of primer, and 0.8 µg of BSA].

The amplifications were carried out in a ICycler© Bio-Rad thermocycler using the following program: 4 min at 95°C followed by 45 amplification cycles (1 min at 94°C, 1 min at 35°C, 1 min at 72°C), with a final extension step at 72°C for 12 min. In every assay, a negative control with all PCR components except for the DNA template was included to detect any contamination. Two sets of 20 primers, each OPA and OPT (Operon Technologies, Alameda, CA) (Table 2) were tested. Amplification products were visualised in 1% agarose gel electrophoresis in 1% TAE buffer stained with ethidium bromide with 1 kb smart ladder (Eurogentec) as a size marker.

Design of locus-specific primers and development of SCAR for biotypes B and Q

The SCAR markers were developed from intense RAPD bands present in all individuals within the same biotype and absent in the other. When the Q biotype DNA was used as template, two specific RAPD bands of 1000 and 900 bp were obtained with the primers OPA-10 and OPT-01, respectively. The two fragments were extracted from the gel and purified with GenElute agarose spin columns (Sigma®). The obtained fragments were amplified with OPA-10 and OPT-01-primers. The fresh PCR product was inserted into TOPO TA Cloning® (Invitrogen) following the supplier's instructions, and transformed into competent *Escherichia coli* strain DH5α. White colonies were grown in LB medium (2 ml) containing 50 mg ml^{-1} of ampicillin. Selected and transformed clones were screened by PCR and the plasmids were purified with QIA Prep Spin Miniprep kit (Qiagen®). The sizes of DNA inserts were checked by *Eco*RI (Roche, Germany) restriction digestion. The inserted DNA fragments of *ca.* 1000 bp was partially sequen-

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1       GTGATCGCAGAGCATTAAGGAGTGTTATATATGGACATTTTGAACTTAAAATGTCAAGTCAAACT
          OPA10

66      AGTGGCGGTTTGGAGTTGGCAATTTCTAAATGTCAATGCGAAAAAAAAAAAATTCCCGTCCGTCAT
        ──────→1000BioQ2D

131     GTTCGGGAGCACAAAATTACCGCCTTAAAATGCGACCTCGCGCTGTCCCAGTGCAACCCCTGCCC

196     TGCCGTCCTCTCAGACTTCTAAGAAAGCCCAATTTTTAGCAACCATCTACAATTTTTTGGAGCAA

261     CATTAACTTTTTCTGCGCGCAATATCGGTTGAAAGATGAAAATAGTTCCATAATTTTCCCAAGAA

326     ACTACCGGGGAAGTGGGAGAAGACTCTAGTTTCTCATAAAAAAAATGAGCACCTGAAAATCAGGG

391     AATGAACATGGTTCAGAAATGTAGACAGAAATTATATTAAATGTATCTATGTATATAAATCTTAA
              1000BioQ2R←──────

456     ATTTTTCAAAACTAAAA
```

[a] positions of the primers are indicated in this sequence

FIG. 1. Partial sequence of RAPD fragment generated with OPA-10[a].

[a] positions of the primers are indicated in this sequence

FIG. 2. Complete sequence of RAPD fragment generated with OPT-01[a].

ced and the fragment of *ca.* 900 bp was entirely sequenced by Automatic Sequencer Global Edition IR_2 DNA Analyser using M13 universal primers. The resulting sequences (Figs 1, 2) were analysed and the corresponding SCAR primers were designed (Table 3). Sequence data were deposited in the EMBL/GenBank data libraries under accession numbers DQ916411 and DQ916412.

In order to amplify SCAR markers, a PCR reaction of 35 cycles was performed. Each cycle consisted of 1 min at 94°C, 1 min at 58 or 60°C (Table 3), and finally 1.5 min at 72°C. The amplified bands were analysed as described for the RAPD amplification products.

RESULTS

Reproducibility of RAPD-PCR

Reproducibility is essential in RAPD amplification. Indeed, results of improved PCR reactions showed no differences in all PCR products, although occasionally variation in the intensities of individual bands was detected. These results showed that single primers of arbitrary sequence can be used to amplify genomic DNA segments, and thus, polymorphisms of *B. tabaci* biotype products can be detected.

Genetic variation of B. tabaci populations

Samples were collected, for comparison, during 2003, 2004 and 2005 from several host plants, namely tomato (*Lycopersicon esculentum* Mill.), lantana (*Lantana camara*), and melon (*Cucumis melo*) (Table 1). RAPD patterns of *B. tabaci* B and Q biotype populations from Valencia (Spain) were used for comparison. The RAPD-PCR products were generated in the same experiment and resolved on the same gel. A considerable variation was observed in the RAPD banding patterns of the *B. tabaci* samples originated

TABLE 2. List of RAPD non-specific primers

Primer Code	Sequence (5′-3′)	GC content (%)	Number of fragments	Size of fragments (bp)	Primer Code	Sequence (5′-3′)	GC content (%)	Number of fragments	Size of fragments (bp)
OPA-01	CAGGCCCTTC	70	–	–	**OPT-01**	GGGCCACTCA	70	8	550-1500
OPA-02	TGCCGAGCTG	70	8	200-1500	**OPT-02**	GGAGAGACTC	60	1	600
OPA-03	AGTCAGCCAC	60	1	1250	**OPT-03**	TCCACTCCTG	60	–	–
OPA-04	AATCGGGCTG	60	5	400-1500	**OPT-04**	CACAGAGGGA	60	4	400-900
OPA-05	AGGGGTCTTG	60	8	200-1500	**OPT-05**	GGGTTTGGCA	60	8	300-1250
OPA-06	GGTCCCTGAC	70	–	–	**OPT-06**	CAAGGGCAGA	60	9	200-1200
OPA-07	GAAACGGGTG	60	6	500-1500	**OPT-07**	GGCAGGCTGT	70	7	300-1200
OPA-08	GTGACGTAGG	60	7	200-2000	**OPT-08**	AACGGCGACA	60	10	300-1500
OPA-09	GGGTAACGCC	70	6	400-1750	**OPT-09**	CACCCCTGAG	70	–	–
OPA-10	GTGATCGCAG	60	10	300-1125	**OPT-10**	CCTTCGGAAG	60	–	–
OPA-11	CAATGCCCGT	60	10	300-1500	**OPT-11**	TTCCCCGCGA	70	–	–
OPA-12	TCGGCGATAG	60	–	–	**OPT-12**	GGGTGTGTAG	70	3	400-600
OPA-13	CAGCACCCAC	60	2	900-1600	**OPT-13**	AGGACTGCCA	70	5	400-1000
OPA-14	TCTGTGCTGG	60	2	1100-2000	**OPT-14**	AATGCCGCAG	60	6	200-1000
OPA-15	TTCCGAACCC	60	6	600-1800	**OPT-15**	GGATGCCACT	60	5	200-1000
OPA-16	AGCCAGCGAA	60	7	400-1800	**OPT-16**	GGTGAACGCT	60	6	500-1500
OPA-17	GACCGCTTGT	60	–	–	**OPT-17**	CCAACGTCGT	60	3	600-1000
OPA-18	AGGTGACCGT	60	10	400-1750	**OPT-18**	GATGCCAGAC	60	2	1500-1600
OPA-19	CAAACGTCGG	60	7	400-2000	**OPT-19**	GTCCGTATGG	60	–	–
OPA-20	GTTGCGATCC	60	6	400-2000	**OPT-20**	GACCAATGCC	60	8	200-1750
Total			**101**					**85**	

–: no amplification

TABLE 3. Designed SCAR primers

Primer	Nucleotide Sequence (5′-3′)	Biotype specificity (technique)	PCR fragment sizes (bp)	Annealing temperature (°C)
900 BioQ1R 900 BioQ1D	CCACTCAAGTTGGTTAGCTCC TAAACAGCGTAAGTAGGGC	B and Q (SCAR)	~ 900 bp + ~ 600 bp of B-type ~ 600 bp of Q-type	60
1000 BioQ2D 1000 BioQ2R	AGTGGCGGTTTGGAGTTGGC CTGTCTACATTTCTGAACCATG	B and Q (SCAR)	~ 900 bp of B-type ~ 400 bp of Q-type	58

from the same location.

Among 40 RAPD primers tested, 31 primers showed amplification, of which 15 produced clear and scorable bands. The number and size of the DNA fragments produced by each RAPD primer are listed in Table 2. These primers were then chosen for biotyping *B. tabaci* populations. When all primers were used 186 bands were scored for a set of 21 female individuals from each population of *B. tabaci*. The bands were in the range of 200-2000 bp.

The RAPD technique described in this study using the OPA-02 and OPA-10 primers indicated that the B and Q biotypes of *B. tabaci* were present (Fig. 3). The other RAPD primers, namely OPA-11, 16, 18, 19, 20 and OPT-01, 07, 08, 12, 14, 15, 16, 20 used for biotyping, were consistently uniform and showed a high reproducibility (data not shown).

It is worth noting that when OPA-02 and OPA-10 were used, the population of *B. tabaci* collected from lantana (*L. camara*) was found to be Q biotype (Fig. 3a, panel A.2, lanes 1-7 and Fig. 3b, panel B.2, lanes 1-7). Two other populations collected from tomato (*L. esculentum*) and melon (*C. melo*) plants were B biotype along with few Q biotype flies (Fig. 3a, b, panels A.1, B.1, lanes 1-7 and Fig. 3a, b, panels A.3, B.3, lanes 1-7, respectively). Results obtained showed that B biotype was the predominant population in north-east of Morocco.

DNA markers of B. tabaci biotypes

The DNA fragment corresponding to RAPD markers generated by OPA-10 and OPT-01 primers was cloned and partially sequenced (Figs 1, 2). Two pairs of primers (Table 3) were designed, based on these SCAR

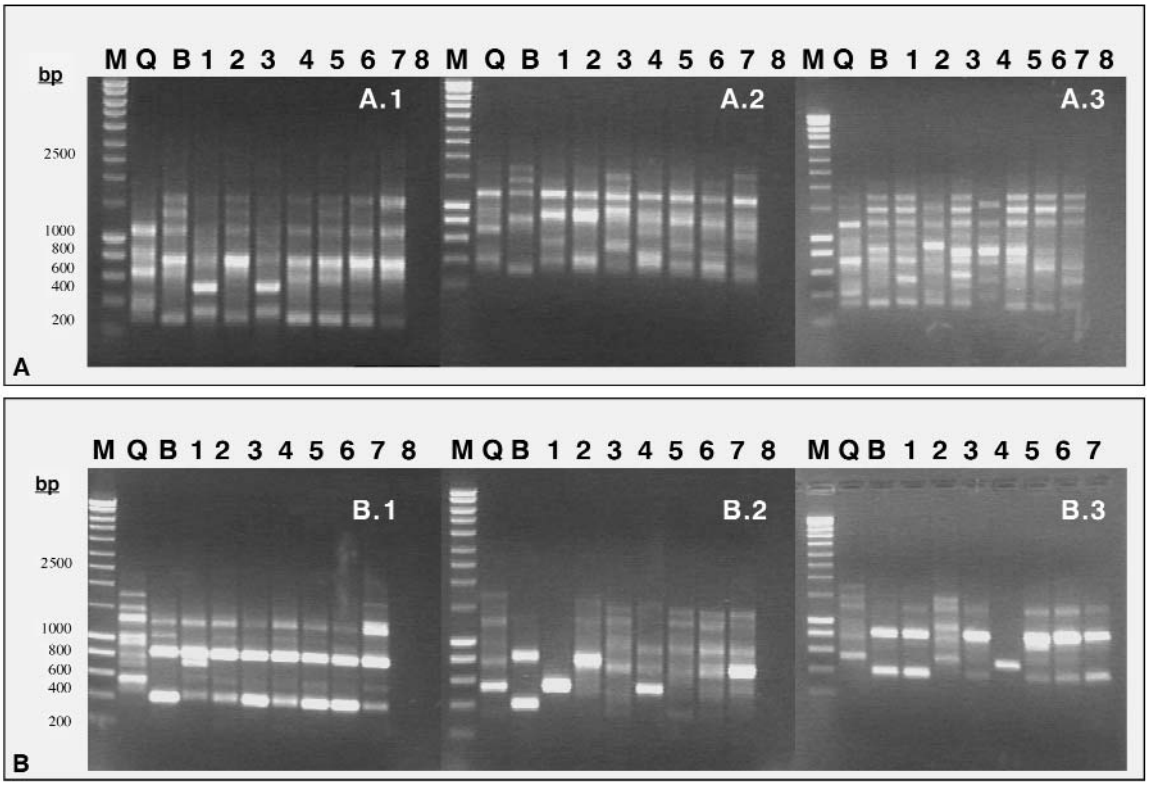

FIG. 3. (A) RAPD patterns produced from *B. tabaci* population using OPA-02. Lane M: smartladder marker 1 kb (Eurogentec). Lane Q: control of Q biotype of *B. tabaci*. Lane B: control of B biotype of *B. tabaci*. Lanes (1 to 7) of A.1: samples of *B. tabaci* population collected from tomato (*Lycopersicon esculentum*). Lanes (1 to 7) of A.2: samples of *B. tabaci* population collected from lantana (*Lantana camara*). Lanes (1 to 7) of A.3: samples of *B. tabaci* population collected from melon (*Cucumis melo*). Lane 8: control without DNA. (B) RAPD patterns produced from *B. tabaci* population using OPA-10. Lane M: smartladder marker 1 kb (Eurogentec). Lane Q: control of Q biotype of *B. tabaci*. Lane B: control of B biotype of *B. tabaci*. Lanes (1 to 7) of B.1: samples of *B. tabaci* population collected from tomato (*L. esculentum*). Lanes (1 to 7) of B.2: samples of *B. tabaci* population collected from lantana (*L. camara*). Lanes (1 to 7) of B.3: samples of *B. tabaci* population collected from melon (*C. melo*). Lane 8: control without DNA.

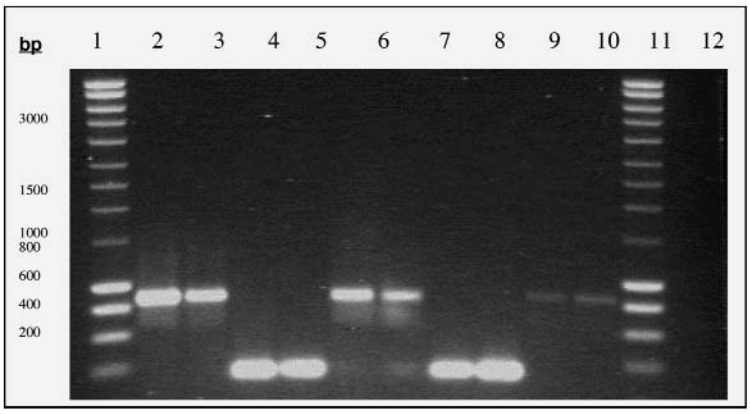

FIG. 4. Sequence characterised amplified regions (SCAR). Analysis based on primer pair 1000BioQ2D/1000BioQ2R. Lanes 1 and 12: smartladder marker 1 kb (Eurogentec). Lanes 2-3: control of B biotype of *B. tabaci*. Lanes 4-5: control of Q biotype of *B. tabaci*. Lanes 6-7: samples of *B. tabaci* population collected from tomato (*L. esculentum*). Lanes 8-9: samples of *B. tabaci* population collected from lantana (*L. camara*). Lanes 10-11: samples of *B. tabaci* population collected from melon (*C. melo*). Lane 13: control without DNA.

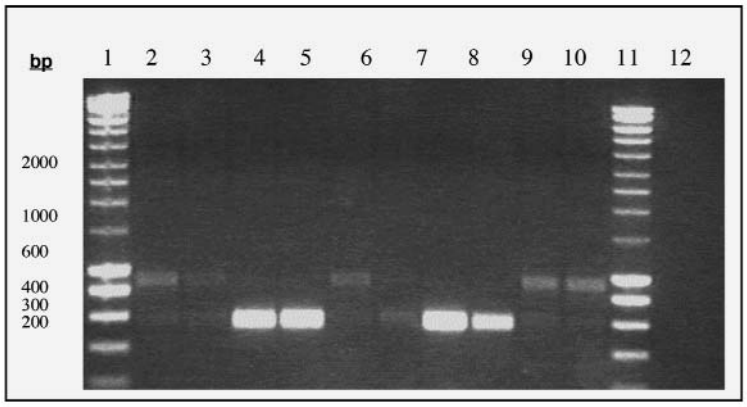

FIG. 5. Sequence characterised amplified regions (SCAR). Analysis based on primer pair 900BioQ2D/900BioQ2R. Lanes 1 and 12: smartladder marker 1 kb (Eurogentec). Lanes 2-3: control of B biotype of *B. tabaci*. Lanes 4-5: control of Q biotype of *B. tabaci*. Lanes: 6-7: samples of *B. tabaci* population collected from tomato (*L. esculentum*). Lanes 8-9: samples of *B. tabaci* population collected from lantana (*L. camara*). Lanes 10-11: samples of *B. tabaci* population collected from melon (*C. melo*). Lane 13: control without DNA.

sequences. Their positions inside the sequence are underlined in Figs 1, 2. The primers generated a B and Q biotype-specific band (Figs 4, 5).

When the primers 1000BioQ2D/1000BioQ2R were used, a single SCAR of *ca.* 400 bp corresponding to Q biotype, and another single SCAR of *ca.* 900 bp corresponding to B biotype (Fig. 4) were obtained. The second pair of primers 900BioQ1D/900BioQ1R yielded a single SCAR of *ca.* 600 bp for Q biotype of *B. tabaci* with a high intensity of the PCR product when compared with the product of B biotype generated with the same primers, and the second band of *ca.* 900 bp with a low intensity for B biotype (Fig. 5).

DISCUSSION

A great effort was made to ensure reproducibility of RAPD analysis and the distinction of *B. tabaci* populations was therefore not based on the evaluation of the unique RAPD fragments (characteristic of a particular population), but on the polymorphic patterns obtained with a number of separate RAPD approaches (Fig. 3, Table 2). It should be noted that RAPD markers were dominant, which resulted in the appearance of a greater band sharing among diploid individuals drawn from a panmictic population that was expected among homozygous lines. Given that *B. tabaci* is a haplodiploid species (males being haploid and females diploid) (Blackman & Cahill, 1998), the fragment pattern of females cannot be formally compared with the corresponding fragment pattern of males (data not shown). Indeed, the obtained profiles for whiteflies from different sexes of *B. tabaci* using the same primer were different (Moya *et al*., 2001). For this reason, analyses of females and males were carried out separately. In contrast, when SCAR markers were used there was no problem to differentiate between biotypes regardless of their sex.

The optimal values for each component were established for RAPD-PCR reaction. The two key reaction-limiting components were high concentrations of DNA and low concentrations of nucleotides. The Mg^{2+} concentration was a key element in determin-

ing the size, distribution and number of RAPD products in the amplification profile. Higher Mg^{2+} availability decreased the number and size of the amplification products in the RAPD profile. Decreasing the Mg^{2+} availability increased the size and number of products. Wolff *et al*. (1993) reported that the concentration of Mg^{2+} required for optimal RAPD amplification was dependent on the primer sequence used. Therefore, maximization of the primer-template interaction required optimisation of Mg^{2+} concentration (Williams *et al*., 1990).

The primers used in this study produced RAPD patterns that distinguished between *B. tabaci* biotypes (Table 2). Results obtained showed that RAPD banding patterns of north-east of Morocco B biotypes were identical to those of Spain confirming the existence of the B biotype in Morocco along with the Q biotype (Fig. 3). While several studies using Moroccan whitefly populations of *B. tabaci* from other locations reported the presence of the Q type (Monci *et al*., 2000; Simon, 2002; Sseruwagi *et al*., 2005; De la Rua *et al*., 2006), the present report is one of the first published ones on the invasion of the B biotype into Morocco (Tahiri *et al*., 2006). The B biotype is located only in the region of north-east of Morocco suggesting that it was probably introduced recently to Morocco, whereas the Q biotype is spread nationwide revealing that this biotype is indigenous or at least has been present for a long time in Morocco. This finding is in agreement with research undertaken on Spanish populations of *B. tabaci* which showed that biotype Q was present before biotype B (Moya *et al*., 2001). The presence of biotype Q has been also reported in other North African countries (Tunisia, Algeria and Egypt) (Benmessaoud-Boukhalfa & Benmessaoud, 2003; De la Rua *et al*., 2006), in the Canary Islands (Beitia *et al*., 1998), Italy (Demichelis *et al*., 2000), and Israel (Horowitz *et al*., 2003). The DNA sequencing of the mitochondrial 16S rRNA and cytochrome oxidase I (COI) genes for the same or similarly representative *B. tabaci* populations indicated that the B biotype was native to Africa or the Middle East (Frohlich *et al*., 1999).

In a RAPD assay, a short (*ca.* ten nucleotides long) arbitrary primer was used, which generally annealed with multiple sites in different regions of the genome and amplified several loci simultaneously. Although the technique is simple, relatively inexpensive and has been employed to analyse the genetic diversity of *B. tabaci*, it generates relatively complex patterns (Guirao *et al*., 1997; Lima *et al*., 2000, 2002). To overcome the reproducibility problem associated with the RAPD technique, RAPD markers were converted into SCAR markers (Paran & Michelmore, 1993).

In the present study, rapid molecular diagnostic tools for the B and Q biotypes of *B. tabaci* were established. First, two RAPD-PCR products, specific to the B and Q biotypes were cloned and sequenced. The resulting sequences did not show a significant homology with any known genes (Figs 1, 2). Nevertheless, a set of specific PCR primers was designed on the basis of these sequences and was found to be useful in distinguishing between B and Q biotypes (Table 3). The two groups revealed with RAPD analysis were confirmed and identified with SCAR markers developed in this study. These designed markers were highly informative in the screening and identification of *B. tabaci* populations (Figs 4, 5). The application of SCAR markers has allowed mapping of the distribution of the two biotypes of *B. tabaci* in Morocco. Therefore, the epidemiology of TYLCV could well be monitored. This methodology could also be used in order to identify the presence of other genotype clusters characterised by particular SCAR haplotypes of the insect with different degree of implication in the epidemics of TYLCV (Agusti *et al*., 1999, 2000).

It is worth noting that few individuals showed patterns that are neither typical of Q nor of B (Fig. 3a, A.1, individuals 1 and 3). More studies are necessary concerning molecular sequences (mitochondrial and nuclear genomes), host range phenotypes and mating compatibility among biotypes and subgroups of *B. tabaci* to elucidate these results.

Reproducibility of the amplification using these SCAR markers was checked by repeating each reaction at least twice without deliberate alteration in the protocol. Ten individual reference samples of *B. tabaci* biotypes B and Q (Valencia, Spain) were screened with the corresponding SCAR (900Bio1R/900BioQ1D; 1000BioQ2D/1000BioQ2R) primer pairs, respectively. The same banding profiles as showed in Figs 4 and 5 were obtained (individuals 2 to 5). Modification of the annealing temperature did not generate an alternate or extra allele other than the SCAR, confirming the specificity of these SCAR markers. These results were consistently present in all the samples collected from the three studied populations of *B. tabaci*.

CONCLUSIONS

In this study, RAPD markers amplified using the primers of OPA and OPT sets distinguished between

the B and Q biotypes of *B. tabaci* in north-east of Morocco. The presence of these two putative biotype-specific RAPD markers in all studied populations led us to design biotype-specific SCAR primers. The latter allowed the amplification of a unique DNA fragment corresponding to the expected biotype under less stringent PCR reaction conditions. Both SCAR markers used in this study indicated that the B and Q biotypes of *B. tabaci* were present in north-east of Morocco. The method described herein is a simple and rapid genotyping assay of *B. tabaci*. This approach is an essential initial step in this process, because the identification of *B. tabaci* biotypes is a key to understand the disease transmitted by viruses carried by the insect (Guirao *et al*., 1997). This method is advantageous because it is reproducible, efficient, and permits the analysis of a large number of samples. The method is also inexpensive and we hope that our approach of developing biotype-diagnostic SCAR markers may pave the way for unambiguous identification of the biotypes B and Q of *B. tabaci*.

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REFERENCES

Agusti N, de Vicente MC, Gabarra R, 1999. Development of sequence amplified characterized region (SCAR) markers of *Helicoverpa armigera:* a new polymerase chain reaction-based technique for predator gut analysis. *Molecular ecology*, 8: 1467-1474.

Agusti N, de Vicente MC, Gabarra R, 2000. Developing SCAR markers to study predation on *Trialeurodes vaporariorum*. *Insect molecular biology*, 9: 263-268.

Bedford ID, Briddon RW, Brown JK, Rosell RC, Markham PG, 1994. Geminivirus transmission and biological characterisation of *Bemisia tabaci* (Gennadius) biotypes from different geographic regions. *Annals of applied biology*, 125: 311-325.

Beitia F, Hernandez-Suarez E, Carnero A, Alonso C, Cenis JL, 1998. Analysis of biotypes of *Bemisia tabaci* (Genn) (Hom: Aleyrodidae) and its parasitoids in the Canary Islands. *Abstracts of the 2nd International Workshop on Bemisia and Geminiviral Diseases, San Juan, Puerto Rico*.

Benmessaoud-Boukhalfa H, Benmessaoud K, 2003. Temporal distribution and natural enemies of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) in Algeria. *Third International Bemisia Workshop, Barcelona*.

Blackman RL, Cahill M, 1998. The karyotype of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Bulletin of entomological research*, 88: 213-215.

Brown JK, 1994. Current status of *Bemisia tabaci* as a plant pest and virus vector in agroecosystems worldwide. *FAO plant protection bulletin*, 42: 3-32.

Calvert LA, Cuervo M, Arroyave JA, Constantino LM, Bellotti A, Frohlich D, 2001. Morphological and mitochondrial DNA marker analyses of whiteflies (Homoptera: Aleyrodidae) colonizing cassava and beans in Colombia. *Annals of the entomological society of america*, 94: 512-519.

Campbell BC, Duffus JE, Baumann P, 1993. Determining whitefly species. *Science*, 261: 1333-1335.

Cervera MT, Cabezas JA, Simon B, Martinez-Zapater JM, Beitia F, Cenis JL, 2000. Genetic relationships among biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae) based on AFLP analysis. *Bulletin of entomological research*, 90: 391-396.

Costa HS, Brown JK, 1991. Variation in biological characteristics and esterase patterns among populations of *Bemisia tabaci*, and the association of one population with silver leaf symptom induction. *Entomologia experimentalis et applicata*, 61: 211-219.

Costa HS, Brown JK, Sivasupramaniam S, Bird J, 1993. Regional distribution, insecticide resistance, and reciprocal crosses between the A and B biotypes of *Bemisia tabaci*. *Insect science and its application*, 14: 255-266.

De Barro PJ, Hart PJ, 2000. Mating interactions between two biotypes of the whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Australia. *Bulletin of entomological research*, 90: 103-112.

De Barro PJ, Driver F, Trueman JWH, Curran J, 2000. Phylogenetic relationships of world populations of *Bemisia tabaci* (Gennadius) using ribosomal ITS1. *Molecular phylogenetics and evolution*, 16: 29-36.

De Barro PJ, Trueman JWH, Frohlich DR, 2005. *Bemisia argentifolii* is a race of *B. tabaci* (Hemiptera: Aleyrodidae): the molecular genetic differentiation of *B. tabaci* populations around the world. *Bulletin of entomological research*, 95: 193-203.

De la Rua P, Simon B, Cifuentes D, Martinez-Mora C, Cenis JL, 2006. New insights into the mitochondrial phylogeny of the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) in the Mediterranean Basin. *Journal of zoological systematics and evolutionary research*, 44: 25-33.

Demichelis S, Bosco D, Manino A, Marian D, Caciagli P, 2000. Distribution of *Bemisia tabaci* (Hemiptera: A-

leyrodidae) biotypes in Italy. *Canadian entomologist*, 132: 519-527.

Frohlich DR, Torres-Jerez I, Bedford ID, Markham PG, Brown JK, 1999. A phylogeographical analysis of the *Bemisia tabaci* species complex based on mitochondrial DNA markers. *Molecular ecology*, 8: 1683-1691.

Gennadius P, 1889. Disease of tobacco plantations in Trikonia. The aleurodid of tobacco. *Elleniki georgia*, 5: 1-3.

Guirao P, Beitia F, Cenis JL, 1997. Biotype determination of Spanish populations of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Bulletin of entomological research*, 87: 587-593.

Horowitz AR, Gorman K, Ross G, Denholm I, 2003. Inheritance of pyriproxyfen resistance in the whitefly, *Bemisia tabaci* (Q biotype). *Archives of insect biochemistry and physiology*, 54: 177-186.

Jebbour F, Abaha L, 2002. Situation of TYLCV in Morocco and diagnostic methods. *European and mediterranean plant protection (Bulletin OEPP),* 32: 17-19.

Jones DR, 2003. Plant viruses transmitted by whiteflies. *European journal of plant pathology*, 109: 195-219.

Lima LHC, Navia D, Inglis PW, Oliveira MRV, de Oliveira MRV, 2000. Survey of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) biotypes in Brazil using RAPD markers. *Genetics and molecular biology*, 23: 781-785.

Lima LHC, Campos L, Moretzsohn MC, Navia D, de Oliveira MRV, 2002. Genetic diversity of *Bemisia tabaci* (Genn.) populations in Brazil revealed by RAPD markers. *Genetics and molecular biology*, 25: 217-223.

Monci F, Navas-Castillo J, Cenis JL, Lacasa A, Benazoun A, Moriones E, 2000. Spread of tomato yellow leaf curl virus Sar from the Mediterranean Basin: presence in the Canary Islands and Morocco. *Plant disease*, 84: 490.

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

Ohmori T, Murata M, Motoyoshi F, 1996. Molecular characterization of RAPD and SCAR markers linked to the *Tm-1* locus in tomato. *Theoretical and applied genetics*, 92: 151-156.

Paran I, Michelmore RW, 1993. Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. *Theoretical and applied genetics*, 85: 985-993.

Peterschmitt M, Granier M, Aboulama S, 1999. First report of tomato yellow leaf curl geminivirus in Morocco. *Plant disease*, 83: 1074.

Rosell RC, Bedford ID, Frohlich DR, Gill RJ, Brown JK, Markham PG, 1997. Analysis of morphological variation in distinct populations of *Bemisia tabaci* (Homoptera: Aleyrodidae). *Annals of the entomological society of america*, 90: 575-589.

Simon B, 2002. Los biotipos de *Bemisia tabaci* (Hemiptera: Aleyrodidae) en la Cuenca Mediterranea. Ph. D. Thesis, University of Murcia, Spain.

Sseruwagi P, Legg JP, Maruthi MN, Colvin J, Rey MEC, Brown JK, 2005. Genetic diversity of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) populations and presence of the B biotype and a non-B biotype that can induce silverleaf symptoms in squash, in Uganda. *Annals of applied biology*, 147: 253-265.

Tahiri A, Sekkat A, Bennani A, Granier M, Delvare G, Peterschmitt M, 2006. Distribution of tomato-infecting begomoviruses and *Bemisia tabaci* biotypes in Morocco. *Annals of applied biology*, 149: 175-186.

Vos P, Hogers R, Bleeker M, Reijans M, Van de Lee T, Hornes M, Friters A, Pot J, Paleman J, Kuiper M, Zabeau M, 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic acids research*, 23: 4407-4414.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic acids research*, 18: 6531-6535.

Wolff K, Schoen ED, Peters-Van Rijn J, 1993. Optimizing the generation of random amplified polymorphic DNAs in chrysanthemum. *Theoretical and applied genetics*, 86: 1033-1037.

Wool D, Gerling D, Belloti AC, Morales FJ, 1993. Esterase electrophoretic variation in *Bemisia tabaci* Genn. (Homoptera: Aleyrodidae) among host plants and localities in Israel. *Journal of applied entomology*, 115: 185-196.