



DNA Markers for identifying *Bemisia tabaci* B and Q biotypes originated from various locations in Israel

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DNA markers for defining *B. tabaci* biotypes

Hybridization

RFLP (Restriction Fragment Length Polymorphism)

PCR-based polymorphism

RAPD-PCR (Random Amplified Polymorphic DNA)

AFLP (Amplified Fragment Length Polymorphism)

Random
PCR-based
polymorphism

DNA sequencing (mtCOI, 16S rDNA, ITS, 18S rDNA)

SSR (Single Sequence Repeats), Microsatellites

SCAR (Sequence Characterized Amplified Regions)

CAPS (Cleaved Amplified Polymorphic Sequences)

Specific
PCR-based
polymorphism

RAPD-PCR

- **Low reproducibility. It is essential to use a control**
- **Useful for diagnosis and simple populational studies**

AFLP

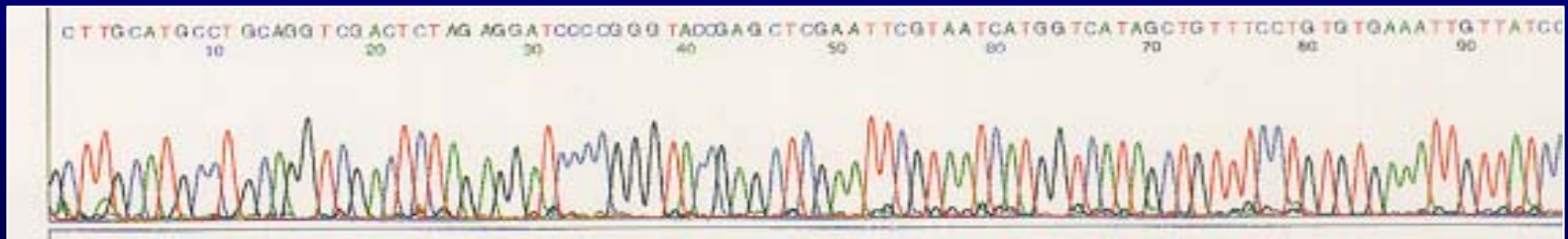
- **High reproducibility and yield**
- **High cost and experimental complication; multilocus, dominant**
- **Useful for populational studies**

SSR

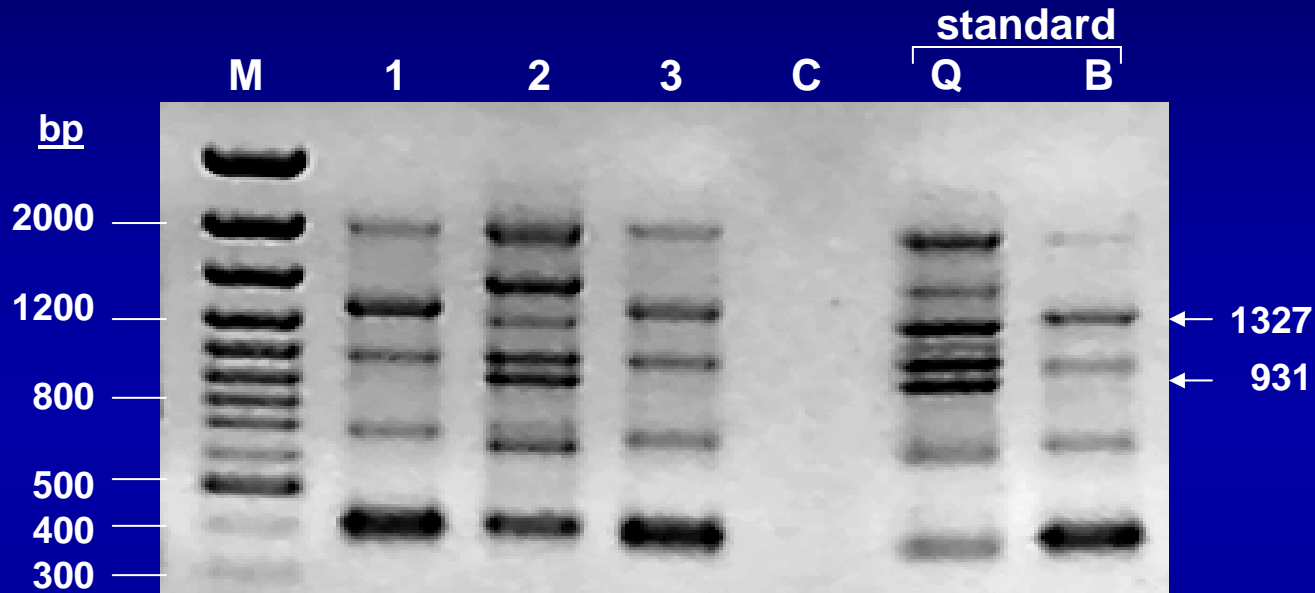
- **High reproducibility, co-dominant**
- **Complicated to develop**
- **Method of choice for populational studies**

DNA Sequencing

- **High reproducibility, simple**
- **High cost**
- **Method of choice for phylogenetic studies**



RAPD-PCR analysis of individual of *B. tabaci* DNA samples with operon primer OPA-06



M, 100 bp DNA Ladder Plus;

lanes 1 & 3, samples obtained from Sde-Eliahu and Beit Dagan;

lane 2, a sample obtained from Hof Carmel;

C, a control without DNA;

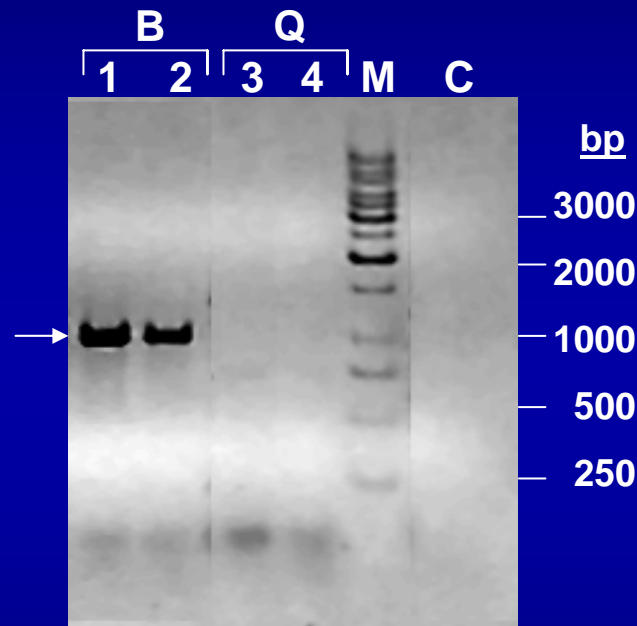
Q, a sample from reference Q biotype from population Pyri-R;

B, a sample from reference B biotype from population Pyri-S.

Arrows mark the position of Q and B specific bands.

Khasdan *et al.* (2005)

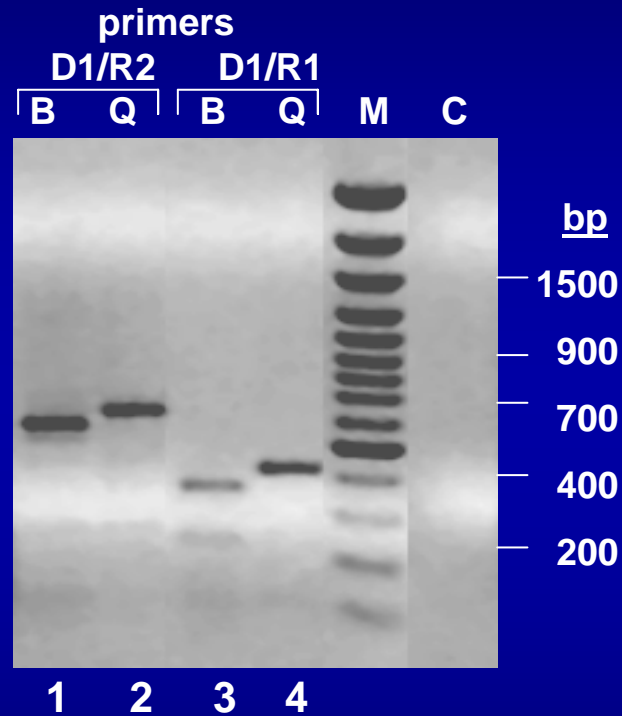
**SCAR (Sequence Characterized Amplified Regions)
analysis based on specific primers to B biotype
(D1-B1 and R2-B1new, designed on basis RAPD-PCR)**



Lanes 1 & 2, samples from population Pyri-S (B biotype);
lanes 3 & 4, samples of populations from Pyri-R (Q biotype);
M, 1 kb DNA Ladder; C, control without DNA.

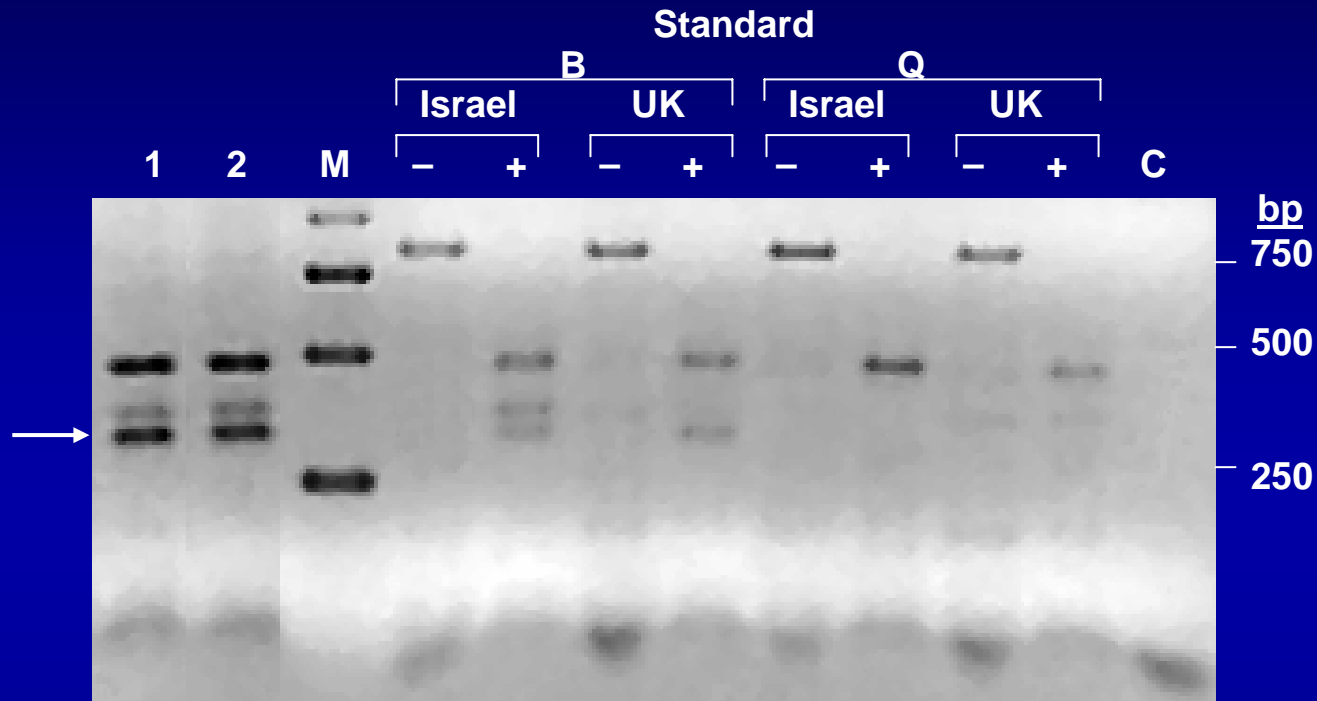
Arrow marks the position of B specific band.

CAPS (Cleaved Amplified Polymorphic Sequences) analysis using different primer combinations, following digestion with *MspI*



D1-Q6 & R2-Q6 primers: lanes 1 & 2, samples from population Pyri-S & Pyri-R, respectively;
D1-Q6 & R1-Q6 primers: lanes 3 & 4, samples from population Pyri-S & Pyri-R, respectively;
M, 100 bp DNA Ladder Plus; C, control without DNA.

CAPS analysis based on primers complementary to the sodium channel gene sequences



Product of PCR (uncut (-) and digested with *AsuI* (+)).

Lanes 1 & 2, samples obtained from Ma`ayan Zevi (Jul 2003) digested with *AsuI*;
M, 1 kb DNA Ladder;

B, samples from reference B biotype from John Innes Center (UK)
and from population Pyri-S (Israel);

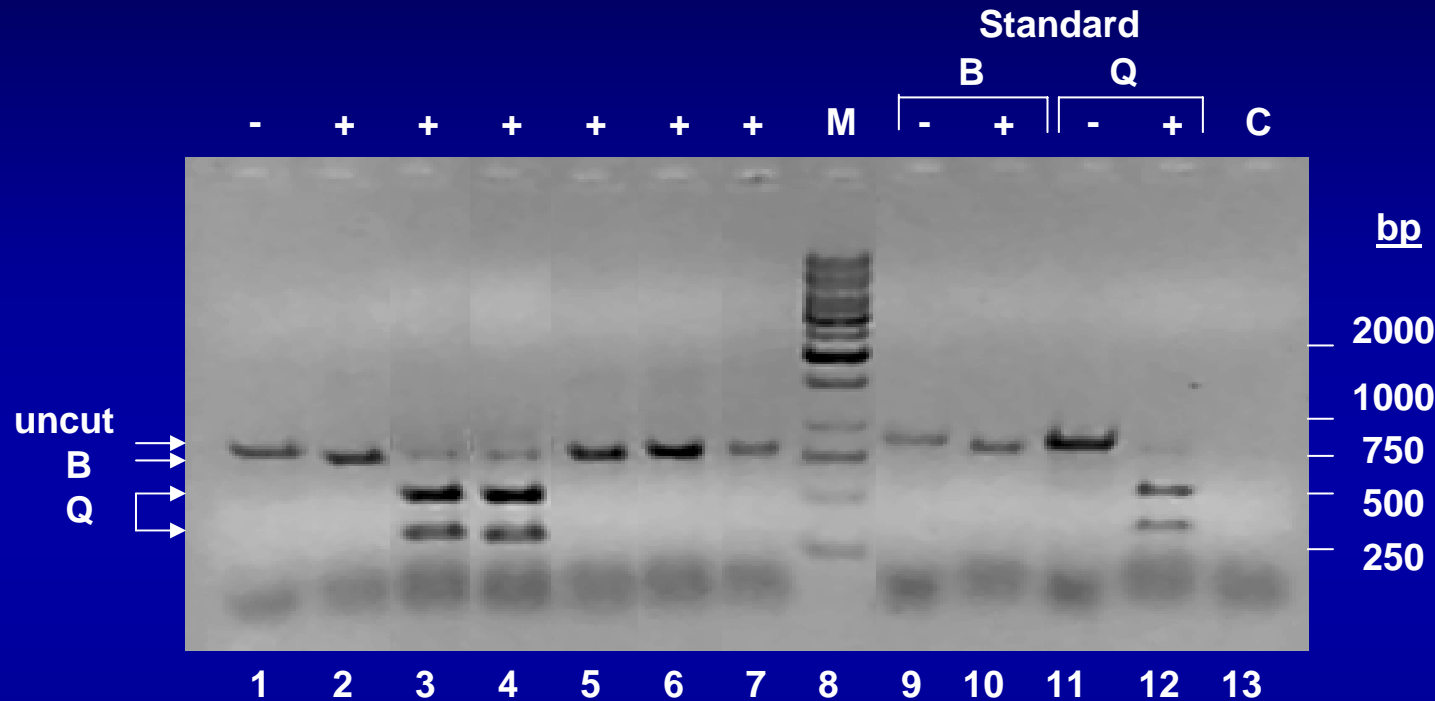
Q, samples from reference Q biotype from John Innes Center (UK)
and from population Pyri-R Israel);

C, a control without DNA;

Arrow marks the position of B specific band.

Khasdan *et al.* (2005)

CAPS analysis based on primers complementary to the mtCOI gene sequences



PCR product (uncut (-) and digested with *Vspl* (+)).

Lanes 1 - 7, samples obtained from Ma`ayan Zevi (Jul 2004);

M, 1 kb DNA Ladder;

B, samples from reference B biotype from CNR (Italy);

Q, samples from reference Q biotype from CNR (Italy) ;

C, a control without DNA;

Arrows mark the positions of uncut PCR products,

B and Q specific bands.

Khasdan *et al.* (2005)

**Comparison of different techniques
for detection of *B. tabaci* biotype (two standard- and
three field-populations were compared using four techniques)**

Population	Technique			
	RAPD-PCR	SCAR and CAPS on basis RAPD	CAPS for sodium channel gene	CAPS for mtCOI sequence
Pyri-S (laboratory)	232 B ^{(a)28}	79 B	55 B	131 B
Pyri-R (laboratory)	177 Q (18)	73 Q	78 Q	143 Q
^b Ma'ayan Zevi (Jul 2003)	20 B	20 B	15 B	20 B
Sha'alvim (Jul 2003)	10 Q	10 Q	10 Q	20 Q
Me'ir Shefe'ya (Jul 2003)	10 B	10 B	10 B	15 B

^aIn parenthesis, undetectable biotype (RAPD-PCR).

^bDNA from the same individuals was used for comparing all the techniques.



Conclusions



- According to the described methods, the B and Q biotypes are present in Israel.
- Comparisons studies that were done with the described methods obtained similar results. Hence, these methods are suitable for rapid high throughput molecular diagnostic for B and Q biotypes of *B. tabaci*.

Thanks!

