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Analysis of whitefly, *Bemsia tabaci*: sequencing of the mitochondrial cytochrome oxidize I mtCoI

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Abstract

Bemisia tabaci (Genn.) is one of the important pest and affects a broad range of agricultural, vegetables and ornamental crops in the world having many biotypes. Since long people have been working on different techniques right from esterase banding to mitochondrial cytochrome oxidize I (mt COI) and internal transcribed spacer (ITS) of ribosomal DNA to work out the phylogeny of whitefly with simple techniques. Whitefly has been described with evidence that *B. tabaci* should be considered a cryptic complex species of 11 well- defined high-level groups containing at least 24 morphologically indistinguishable species and this finding was based on the mitochondrial cytochrome oxidize I (mtCO I) sequence. In present study, the mtCO I sequence of *B. tabaci* was analyzed from population samples collected from different host plants - Potato (Solanum tuberosum), brinjal (Solanum melongena) and lady's finger (Abelmoschus esculentus) from district Meerut, Uttar Pradesh, India. Results obtained showed that amplification of mtCO I gene fragment using the primers (C1-J-2195 and L2-N-3014) produced *B. tabaci* specific ~800bp band in all the samples of whitefly. Further Sequencing, homology search by Blast and comparison against the consensus sequences of Dinsdale or revealed that these samples belong to group Asia II 1which includes K, P, ZHJ2, PCG-1, SY and PKI, respectively.

Keywords: whitefly, biotypes, mitochondrial cytochrome oxidize I

1. Introduction

The globally important (Boykin *et al.*, 2007; Dinsdale *et al.,* 2010) phloem-feeding insect, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) lives predominantly on herbaceous species. It is a pest of ornamental, vegetable, grain legume, and cotton, causing damage directly through feeding (Oliveira *et al.*, 2001) and indirectly through the transmission of plant pathogenic viruses, primarily begomoviruses and resulting many diseases (Jones, 2003).

Bemisia tabaci is a species complex of about 41 biotypes (De Barro *et al.*, 2011) in theses biotypes many biotypes represent different cryptic species that cannot be identified by morphological traits (Rosell *et al.*, 1997).These biotypes have been reported to have different biological characteristics with respect to invasiveness, insecticide resistance profile (Horowitz *et al.,* 2003), pathogen vector and host range (Bedford *et al.*, 1994) creating a need to have precise biotype determination methods. Molecular markers have been used to study the genetic polymorphism of *B. tabaci* and mtCOI gene is the most widely applied DNA region for determine the genetic structure of *B. tabaci* (Dinsdale *et al.*, 2010; De Barro *et al.*, 2011; Frohlich *et al.*, 1999).On the basis mtCOI gene different biotypes have been identified from South-East Asia. Biotypes K from Pakistan and H from India were reported by Bedford and co-workers in 1992. In India, other than biotype B from south India biotype G was assigned to a populations on watermelon from Kerala and Biotype H was the designation given to whiteflies collected on watermelon from Kerala and to a population on cotton, eggplant and potato collected in Gujrat and biotype 'I' from Maharastra (Brown *et al.* 1994). In the recent past, whitefly have been causing damage to many crops in north India hence there was a need to have precise information on biotype present in this part of country and its determination with simple method.

In the present study sequencing the mitrochondrial cytochrome c oxidase I (mtCO I) gene was used as it is the more reproducible and informative method to determine the genetic affiliation of a *B. tabaci* populations(De Barro *et al.*, 2011).

2. Material and Methods

Adults of *B.tabaci* were collected from 12 localities in the Meerut District, during 2012-2013, using a test tube and preserved immediately in 95 % ethanol at -20^0 C until processing. Samples were collected mostly from potato and brinjal. A single step DNA Extraction method was used (Lima *et al.*, 2002) with some modifications. Each individual female whitefly was homogenized in a 1.5 ml micro centrifuge tube with 70 µl of lysis buffer (10 Mm Tris–HCl PH 8.0; 1 mM EDTA; 0.30% Triton X-100, 60 µg/ml Proteinase K). The homogenate was then incubated at 65^{0}C for 45 Minutes. Samples were then boiled for 5 minutes to inactivate the proteinase K and incubated at -20^{0}C for 10 min. Cell debris and Proteins were removed by centrifugation for 3 min at 13,000 rpm. After centrifugation, the supernatant was transferred to a new tube and stored at -20^{0}C.

The primers used were- Part of mtCOI **(**Frohlich *et al.*, 1999)

C1-J-2195-5'TTGATTTTTGGTCATCCAGAAGT3'

L2-N-3014-5'TCCAATGCACTAATCTGCCATATTA3'

PCR was carried out on a thermal cycler (Eppendrof Master Cycler PCR, Hamburg (Germeny) with the following cycling parameters; 94^0C for 2 min as initial denaturation followed by 30 cycles of 94^0C for 30 Sec; 52^0C for 30 Sec; 72^0C for 1 min and 72^0C for 10 min as final extension. PCR was performed in 25 µl total reaction volume containing 1 µl of 20 picomoles of each primer forward and reverse, 2.5 µl of 10 X buffer contain 20 mM $MgCl_2$, 2.5µl d NTP (2 mM each), 0.2µl (5 U/µl) of Fermentas Dream Taq Polymerase, 15.8 µl of autoclaved water and 2 µl of DNA template. The amplified products were resolved in 1.5% agarose gel, stained with Ethidium bromide (10µg/ml) and visualized in a gel documentation system. PCR mix and PCR cycling parameter were the same except the annealing temperature and 6% DMSO was used in mixture for ITS1 region. The amplified DNA was purified using following manufacturer instructions (gene jet gel extraction kit).

The PCR amplified fragments were eluted and sequencing was carried out in an automated sequencer (ABI prism ® 3730 XL DNA analyzer; Applied biosystems, Maryland, USA) using universal primers both in forward and reverse directions. Homology search was carried out using Blast (http://www.ncbi.nlm.nlh.gov) and the difference in COI sequences of *B.tabaci* were determined using the sequence alignment editor Bio Edit version (10.7) and compared against the consensus sequences of Dinsdale *et al*., (2010). The alignment was further analyzed using the MEGA 5.0 program, using the Neighbor–joining method with a "bootstrap" value of 1000.

Table 1- *Bemisia tabaci* collected from different hosts and localities

• Sample	• location	• Geographical • Coordinates	• Host plant	• Collect
• 1	• Modipuram	• 28.99^0 N/77.70^0 E	• Potato	• Oct/12
• 2	• Rohta	• 29.34^0 N/77.31^0 E	• Potato	• Jul/12
• 3	• Sardhana	• 29.15^0 N/77.62^0 E	• Egg plant	• Sep/12
• 4	• Daurala	• 29.11^0N/77.70^0E	• Potato	• Oct/12

3. Results and Discussion

In India there is a high incidence of apical leaf curl virus disease (ALCV) in many crops by both non-silverleaf (north India) and silver leaf (on squash) inducing (south India) populations of whitefly. The population collected from Modipuram on potato was not biotype B (Malik and Singh, 2007). Again samples of adult whitefly were collected (2008-09) from Modipuram (Meerut, UP) from cotton, moongbean and cucumber in the first week of September just before the onset of potato planting season and by amplification of mtCO I gene fragment from whitefly populations Dr. Brown placed it in Asia-1 a of Asia-Pacific Indian subcontinent group (fig. 2).

Results of present study revealed that amplification of mtCO I gene fragment using the primer (C1-J-2195 and L2-N-3014) produced *B. tabaci* specific ~800bp band in all the samples of whitefly (Fig.1). Samples collected belonged to Asia II_1 by using consensus sequences to assign group affiliation (Dinsdale *et al.*, 2010) (fig. 3).The differences noted were as shown in table (2).

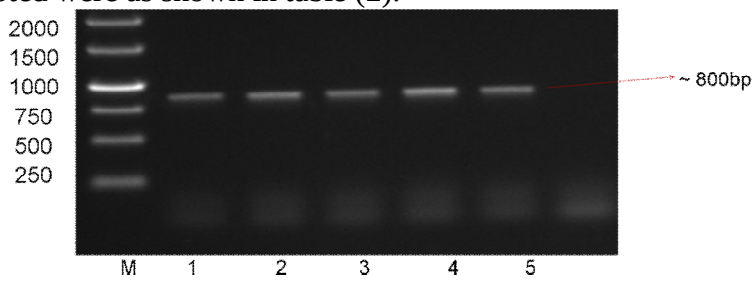

Fig. 1. Amplification of *mt*COI gene fragment using the universal primer (C1-J- 2195 and L2-N-3014). M: DNA Ladder; (1-2-3) potato; (4) brinjal; (5) lady's finger.

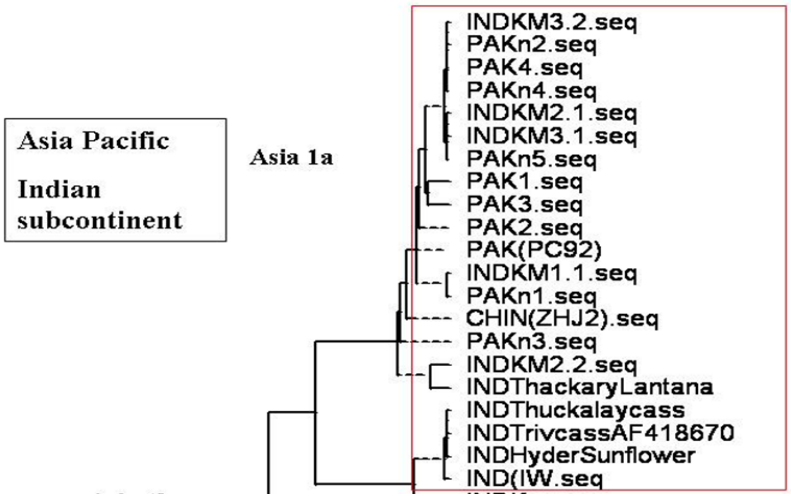

Fig. 2. Mitochondrial Cytochrome oxidase I tree of *B. tabaci* containing Indian samples INDKM 1, 2 and 3 of Modipuram (Meerut) (courtesy Dr. J K Brown)

Table No 2: Nucleotide variability in mtCOI gene:

Sample/bp position	**82**	**124**	**251**	**271**	**424**	**442**	**452**	**475**	**580**	**660**	**712**
A	A	T	C	C	A	C	G	C	T	T	G
B	A	T	T	T	A	T	G	C	C	T	G
C	G	G	C	T	G	T	T	T	T	T	A
D	A	T	C	C	A	C	G	C	T	C	G

On the basis of mt COI, Asia 1had been previously referred to as 'Indian Subcontinent' (Brown, 2001), 'China, Pakistan and Thailand cluster' (Viscarret *et al.*, 2003), 'Southeast Asia/Far East' (Berry *et al.*, 2004) and 'Group 4' by Perring (2001). On the basis of comparison, mt COI biotypes were mapped to Asia -1(H, M), Asia II

_1 (K, P, PCG-1, PK1, SY and ZHJ2) and Middle East- Asia Minor 1 (B &B2-squash silver leaf inducing population) (Dinsdale *et al*., 2010).

The possible taxonomic confusion for Asia II _1 (Dinsdale *et al*., 2010; De Barro *et al*., 2011) which includes biotypes K, P, PCG-1, ZHJ2, PK1 and SY was explained that in the case of biotypes K and P there was slight difference in esterase banding patterns and Subsequent examination of their mtCO1 showed <2% sequence variation (Bedford *et al*., 1994; De barro *et al*., 2011). ZHJ2 was identified as another biotype, but without comparison to material from Pakistan and Nepal where P, K were obtained. ZHJ2 and K had an identical mt CO1 (De Barro *et al*., 2011). Similarly, biotypes PCG-1, PK1 and SY were all raised without reference to K, P and ZHJ2 and again have mt CO1 that were either identical to K or show <2% mtCO1 sequence divergence (De Barro *et al*., 2011). In all cases there is no data showing biological differences and the identifications have been based solely on molecular data of one form or another (De Barro *et al*., 2011).

Mt COI from PK1 shows <2% variation from what was described as the PCG-1 biotype as well as with those biotypes referred to as K, P, SY and ZHJ2. The rule set developed by Dinsdale *et al*., (2010) removes this ambiguity and shows that all six biotypes are more than likely the same entity. Hence the biotypes identified from potato and egg plants were biotype P which belonged to Asia II 1, a low level group of De Barro and co-workes (2011).

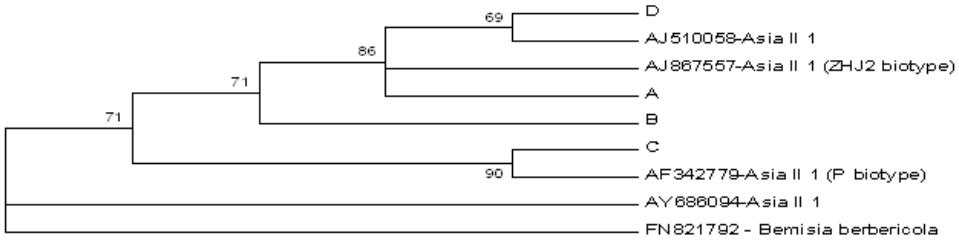

Fig.3 Phylogenetic tree using the mitrochondrial COI gene of *Bemisia tabaci* samples collected in the Meerut District compared with some sequence of GenBank. *Bemisia berbericola* (FN821792) was utilized as outgroup.

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