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Mitrochondrial Cytochrome Oxidize I (mt COI) Diagnostic Technique for the Quick Identification of Biotypes Whitefly, *Bemisia Tabaci* (Gennadius) on different Crops

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ABSTRACT

Bemisia tabaci (Genn.) is one of the important pest and affects a board range of agricultural crops in the world having many biotypes. There is evidence that *B. tabaci* should be considered a cryptic complex species of 11 well-defined groups containing at least 24 morphologically indistinguishable species. Its populations differ biologically with respect to insecticide resistance, virus transmission and host range. Therefore, understanding genetic variation among populations is important for management. In present study, the mtCO I sequence of *B. tabaci* was analyzed from population sample collected from different host plants from different area, Uttar Pradesh. 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 Ellango, revealed that these samples belong to group Asia II. It is highly probable that these all biotypes are the same entity, but have been assigned different names either as a consequence of the use of different methods of identification or a failure to broadly consider the available data in sources such as Genebank.

Keywords : Amplification, Biotypes, COI, *Bemisia tabaci*

I. INTRODUCTION

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

Bemisia tabaci complex is a cryptic species complex, composed of >35 morphologically indistinguishable species (Calvert *et al.,* 2001), varies considerably in its ability to transmit geminiviruses (Bedford *et al.,* 1994), rate of development (Wang & Tsai, 1996) and ability to utilize different host plants (Brown & Bird, 1995), but genetically distinct groups (Boykin *et al.,* 2007 and Dinsdale *et al.,* 2010), molecular (Xu *et al.,* 2010) and biological data 5 supports their status as different species (De Barro *et al.,* 2011). *B. tabaci* has a high intra specific biological and genetic variability (De Barro *et al.,* 2000). *Bemisia tabaci* (Hemiptera: Aleyrodidae) is a species complex of about 41 biotypes (De Barro *et al.,* 2011) out of which 5 (H, P, K, G, B) have been reported from India. It is vector of Apical Leaf Curl Virus (ALCV) in agricultural ecosystems. Among the biotypes, B biotype is most significant having the highest transmission efficiency for ALCV diseases. Biotype B, because of its global pest status, has been the focus of considerable research over the past many years. Previous studies of Genetics structure of *B .tabaci* in India were limited to samples collected from different regions only (Perring et al., 2001, Banks et al., 2001; Rekha et al., 2005, Lisha et al., 2003, Malik and Singh., 2007 Reddy et al., 2012, Singh et al., 2012 and Swati tomar et al., 2014). 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* (Ellango, *et al*., 2015; Dinsdale, 2010; De Barro *et al.,* 2011;

Frohlich *et al.,* 1999). Molecular techniques are especially useful for studying and monitoring mixed populations where biotype B and other biotypes co-exist. Therefore, we aim to present more unified and through account of the current genetic structure of *B. tabaci* in Uttar Pradesh. We examine the genetic identity of *B. tabaci* with a view to determine whether identity may be associated with differences in disease incidence. We have used the mt COI marker gene for this poupose.

II. MATERIAL AND METHOD

Adult whiteflies (230 samples) were collected from different district and analyzed at Central Potato Research Institute Campus Modipurm (Meerut) from 4 distinct host plants ie. Potato, brinjal, Cotton and Tomato and preserved immediately in 100% ethanol.

DNA Extraction, PCR amplification and Sequencing

A single step DNA Extraction method was used (De Barro and Driver, 1997 and 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 65^0C for 45 Minutes. Samples were then boiled for 5 minutes to inactivate the proteinase K and incubated at -20^0C 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 -20^0C. Part of mt COI gene was amplified with primers C1-J-2195- FW (5' TTGATTTTTGGTCATCCAGAAGT 3') and L2-N-3014 RV(5'TCCAATGCACTAATCTGCCATATTA3') (Frohlich *et al.,* 1999). 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. 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 BioEdit 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.

III. RESULT AND DISCUSSION

A. Mitrochondrial cytochrome c oxidase I (*mt* COI) PCR amplification Primer pair specific for *B. tabaci*:

The mitrochondrial cytochrome c oxidase I (*mt*COI) sequence has been used extensively in whitefly identification. Although several primers have been described for amplification of *B. tabaci* mt COI fragments, the most common PCR primers to use for this work were developed for insects by Simon *et al.,* (1994) and first used for *B. tabaci* by Frohlich *et al.,* (1999). Results revealed that amplification of mt COI gene fragment using the primer (C1-J-2195 and L2-N-3014) produced *B. tabaci* specific ~800 bp band in all the samples of whitefly.

Dinsdale *et al*., (2010) have recently proposed that *B. tabaci* should be considered as a cryptic species complex comprising 11 groups containing 24 species. The more reproducible and informative method available to determine the genetic affiliation of a *B. tabaci* population is sequencing the mitrochondrial cytochrome c oxidase I (mt COI) gene (De barro *et al*., 2005) and use consensus sequences to assign group affiliation (Dinsdale *et al*., 2010). Using the nomenclature of Dinsdale *et al*. (2010), the results revealed putative species. Asia II 1 (also referred to in the literature as

biotypes K, P, PCG-1, SY, ZHJ2 and PKI) was recorded from different region.

The *B. tabaci* dataset consisted of global sampling of 570 individuals of mtCOI region (De Barro et al., 2011).Using the nomenclature of Dinsdale *et al*. (2010), the results revealed putative species Asia II 1 (also referred to in the literature as biotypes K, P, PCG-1, SY, ZHJ2 and PKI) was recorded from different region. It is highly probable that these six biotypes are the same entity, but have been assigned different names either as a consequence of the use of different methods of identification or a failure to broadly consider the available data in sources such as Genebank. Moreover, Asia II 1 was associated with high incidences of ALCV, whereas regions where Middle East-Asia Minor 1 was present had a lower incidence. Asia II 1 was recorded from Punjab cotton plants, whereas Asia 1 (also referred to in the literature as biotypes H, M, NA) was found in both Pakistan and Punjab. Three biotypes identified from India biotype G from Kerala, biotype H from Gujarat and biotype I from Maharastra (Brown *et al.,* 1995)**.** A good example of the taxonomic confusion is Asia II 1 (Ellango *et al*., 2015; Dinsdale *et al*., 2010; De Barro *et al*., 2011) which is also known as biotypes K, P, ZHJ2, PK1 and SY. In this case biotypes K and P were raised on the basis of slight differences in esterase banding patterns. 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; in fact, ZHJ2 has an identical to mtCO1 to K (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 mtCO1 that are either identical to K or show <2% mtCO1 sequence divergence (De Barro *et al*., 2011).

We have therefore adopted the Dinsdale terminology in this study (Dinsdale *et al*., 2010; De barro *et al*., 2011). In India the province, which has a high incidence of PALCV (Garg *et al*., 2001), two indigenous species, Asia 1 (also identified in the literature as biotypes H, M and NA), Asia II 1 and Middle East- Asia Minor 1 occur (Simon *et al*., 2003). There are a number of possible explanations. Xu *et al*., (2010) has shown that Asia II 1 and Middle East-Asia Minor 1 are reproductively incompatible. Middle East-Asia Minor 1 has shown to be particularly adept at displacing other members of the species complex (Liu *et al*., 2007). However, the consequence of competition between this species and Asia II 1 is unknown, but one might speculate from their neighboring geographic distributions that neither is sufficiently capable of displacing the other. De Barro *et al*. (2005) showed that local abundance may be sufficient to exclude establishment of an invader, and so if Asia II 1 and Middle East-Asia Minor 1 are indeed well matched, then regional abundance may be sufficient to prevent invasion and displacement. Alternatively, insecticide resistance has been shown to influence capacity by different members of complex to invade and displace other congener members Crowder *et al*., (2010). The rule set developed by Dinsdale *et al*., (2010) removes this ambiguity and shows that all biotypes are more than likely the same entity.

IV. CONCLUSION

Whitefly population has similarities to Asia II 1 population. The distribution patterns of *B. tabaci* suggest that most diversity is present in northern India. *B tabaci* is not known to fly long distance; hence, it was expected that nearby fields of a particular locations would contain the same genetic groups. In contrast, more than one genetic group of *B. tabaci* was sometimes found in same locations, whereas Asia II 1 was found in all neighboring locations.

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Table1. Details of field survey for collection of *B. tabaci* samples across Uttar Pradesh and Rajasthan

Sr. No.	State	District	Field	Host plants	*Bemisa tabaci* Genetic group	GeneBank Accession number (from NCBI)
1.	Uttar Pradesh	Aligarh	I	Brinjal	Asia II 1	AF 342779
			II	Cotton	Asia II 1	AJ 510058
			III	Potato	Asia II 1	AF 342779
			IV	Tomato	Asia II 1	AJ 867557
2.	Uttar Pradesh	Bijnore	I	Brinjal	Asia II 1	JX 993210
			II	Cotton	Asia II 1	JX 993211
			III	Potato	Asia II 1	JX 993215

			IV	Tomato	Asia II 1	JX 993218
3.	Uttar Pradesh	Roorkee	I	Brinjal	Asia II 1	JX 993204
			II	Cotton	Asia II 1	JX 993205
			III	Potato	Asia II 1	JX 993220
			IV	Tomato	Asia II 1	JX993219
4.	Uttar Pradesh	Muzaffarnagar	I	Brinjal	Asia II 1	JX 993232
			II	Cotton	Asia II 1	JX 993230
			III	Potato	Asia II 1	JX 993229
			IV	Tomato	Asia II 1	JX993228
5.	Uttar Pradesh	Modinagar	I	Brinjal	Asia II 1	JX 993217
			II	Cotton	Asia II 1	JX 993209
			III	Potato	Asia II 1	JX 993207
			IV	Tomato	Asia II 1	JX 993202
6.	Uttar Pradesh	Hastinapur	I	Brinjal	Asia II 1	JX 993213
			II	Cotton	Asia II 1	JX 993212
			III	Potato	Asia II 1	JX 993206
			IV	Tomato	Asia II 1	JX 993208
7.	Uttar Pradesh	Hapur	I	Brinjal	Asia II 1	JX 992302
			II	Cotton	Asia II 1	JX 993180
			III	Potato	Asia II 1	JX 993182
			IV	Tomato	Asia II 1	JX 993184
8.	Uttar Pradesh	Amroha	I	Brinjal	Asia II 1	JX 993178
			II	Cotton	Asia II 1	JX 993190
			III	Potato	Asia II 1	JX 993192
			IV	Tomato	Asia II 1	JX 993189
9.	Uttar Pradesh	Ghaziabad	I	Brinjal	Asia II 1	JX 993200
			II	Cotton	Asia II 1	JX 993186
			III	Potato	Asia II 1	JX 993199
			IV	Tomato	Asia II 1	JX 993197
10.	Rajasthan	Jodhpur	I	Brinjal	Asia II 1	JN 410778
			II	Cotton	Asia II 1	JN 410779
			III	Potato	Asia II 1	JN 410801
			IV	Tomato	Asia II 1	JN 410800