Genetic diversity of whitefly, *Bemisia tabaci* population of Rajendranagar, Telangana, India

KP Manju, K Vijaya Lakshmi, Ch. V Durga Rani, B Sarath Babu and K Anitha

Abstract

The genetic identification of whiteflies collected from the different fields of Rajendranagar, Hyderabad, Telangana, India during summer 2017 was done by SSR-PCR of the mitochondrial cytochrome oxidase I (mtCOI) gene. The results obtained from the present study revealed the amplification of mitochondrial cytochrome oxidase I (mtCOI) gene fragment using the primers (C1-J-2195 and L2-N-3014) produced *Bemisia tabaci* specific ~860bp band in all the samples of *B. tabaci*. MtCOI gene of this *B. tabaci* samples collected from Rajendranagar, Telangana were compared with other known sequences in GenBank and the test samples showed high level of nucleotide and amino acid identity with Asia I and Asia II-I genetic groups. The phyllogenetic tree constructed based on UPGMA (Unweighted Pair Group Method with Arithmetic Mean) analysis with 10 mtCO1 sequences of *B. tabaci* samples also revealed that Rajendranagar *B. tabaci* population clustered with sequences corresponding to Asia-I and Asia II-I genetic groups. So far there is no report on the presence of Asia II-I genetic group from the Telangana state.

Keywords: *Bemisia tabaci*, mitochondrial cytochrome oxidase, sequencing, phylogenetic tree, genetic groups

Introduction

Okra (*Abelmoschus esculentus* L.), commonly known as bhendi, belongs to the family Malvaceae is one of the important vegetable crops cultivated throughout India. The production and quality of okra fruits are affected by an array of sucking and fruit boring pests from sowing until harvest. The key sucking pests of okra are whiteflies, aphids, jassids, thrips and mites. Among the sucking pests, *whitefly, Bemisia tabaci* Gennadius causes economic damage to okra by feeding on phloem sap, there by contaminating leaves and fruits with honey dew that causes sooty mould formation [8].

The whitefly, *Bemisia tabaci* is quite a devastating pest on okra, cotton, brinjal, tomato and several ornamental plants [9]. Besides causing direct damage, it also transmits an economically important viral disease caused by *Okra yellow vein mosaic virus* (OYVMV) resulting in significant yield losses in okra especially when it occurs in the early stages of crop growth. As compared to healthy plants, diseased plants showed a reduction of 24.9% in plant height, 15.5% decrease in root length, and 32.1% in number of fruits per plant, whereas stem growth was reduced by 16.3% [15].

OYVMV’s family Geminiviridae has a wide variety of plant viruses that wreaks havoc in farms and plantations worldwide resulting in heavy crop losses. In India, survey conducted in okra farms of the states of Karnataka, Andhra Pradesh, Tamil Nadu, Kerala, Maharashtra, Haryana, Uttar Pradesh, Delhi, Chandigarh and Rajasthan showed OYVMV causing disease incidents in 23.00 to 67.78 percentages of crops [16, 17].

OYVMV is transmitted by *B. tabaci*. The debate on the taxonomic status of *B* tabaci species is an ongoing one. Various morphological, behavioral and genetic studies have come up with conclusions that don’t converge on its taxonomic status - resulting in alternative designations such as biotypes and genetic groups for its different populations. Various molecular methods were used to fingerprint *B. tabaci* populations, but it was by using mtCO1 marker genes the scientific community got real insight into *B. tabaci* genetic variability. Its population was categorized into 12 major genetic groups after applying Bayesian phylogeny to mtCO1 gene sequences [3]. Further classifications resulted in 24 cryptic species within 3.5% genetic divergence [3]. In India, very minimal work has been done to categorize *B. tabaci* using...
genetic markers [6, 10], and no work has been done to identify and categorize them using mtCOI gene markers. The decline in the production of okra in India was attributed to several factors such as loss of resistance to BYVMV in ruling varieties [11], emergence of new whitefly genetic groups and development of moderate to high resistance by the whitefly vector to commonly used insecticides [11]. In order to develop sustainable vector associated viral disease management practices for okra cropping system, there is a need to concentrate the studies on the identification of genetic groups of whitefly vector and screening of new okra germplasm for vector and virus resistance.

Materials and Methods
Collection and storage of whiteflies
Whitefly adults were collected from the different fields of Rajendranagar, Hyderabad, Telangana state (five whitefly adults from each field) with the help of aspirator and preserved in 99 per cent ethanol and then stored in the refrigerator prior to molecular analysis.

DNA isolation
Total DNA was extracted from individual B. tabaci adult specimens using DNeasy blood and tissue genomic DNA purification kit (QIAGEN, Hilden, Germany). After removing from ethanol, the whiteflies were washed with double-distilled water to remove alcohol. The individual whiteflies were homogenized in 180 µl ATL buffer (genomic digestion buffer) using a 1.5 ml microcentrifuge tube and micropipette (homogenizer). Then 20 µl of proteinase K and 200 µl AL buffer (genomic lysis buffer) were added and incubated at 56 ºC until the tissue was completely lysed. After that immediately 200 µl absolute ethanol was added and the mixture was transferred (including any precipitate) into the DNeasy Mini spin column placed in a 2 ml collection tube. Samples were centrifuged at about 6000 rpm for 1 min, and the flow-through and collection tubes were discarded. Subsequently wash buffers were added into the spin column and finally 30 µl buffer AE (genomic elution buffer) was added to elute genomic DNA from the column. After 1 min incubation at room temperature, samples were centrifuged at about 8000 rpm for 1 min, and the eluted genomic DNA were directly used for PCR [7].

SSR-PCR analysis
The genetic identification of whiteflies was done by SSR-PCR of the mitochondrial cytochrome oxidase I (mtCOI) gene from Bemisia tabaci. Mtco1 gene was amplified using forward primer 5'-TTGATTTTTTGGTCATCCAGAAGT-3' (C1-J-2195) and reverse primer 5'-TCCAATGCACTAATCTGCCATATT-3' (L2-N-3014) as suggested by Ellango et al. (2015). The components such as sterile distilled water, Taq buffer (10x), dNTP (1mM), forward primer (5pM), reverse primer (5pM), Taq polymerase(1u/µl) and DNA template were added to prepare reaction mixture in a volume of 12 µl, 2 µl, 2 µl, 1 µl, 1 µl, 0.5 µl and 3 µl, respectively to make a final reaction volume of 20 µl. The PCR plate was then set up in a programmable thermal cycler for DNA amplification. PCR was performed with an initial denaturation at 94 ºC for 30 s followed by 35 cycles of 94 ºC for 30s, 50 ºC for 30 s, 72 ºC for 40 sec, and a final extension at 72 ºC for 5 min. After completion of the PCR, the samples were collected and stored at -20°C. Later the products were resolved on 1.5% agarose gel.

Sequence and phylogenetic analysis
The PCR product was cloned into pGEM-T Easy sequencing vector (Promega, Madison, Wisconsin, USA). Plasmids from positive transformants were sequenced at Nuziveedu Seeds Pvt Ltd (NSL), Hyderabad. DNA sequences were aligned and compared with the sequences similarity with other known sequences using BLAST (Basic Local Alignment Search Tool) at the National Centre for Biotechnology Information (NCBI) website. Phylogenetic relationships were inferred by Molecular Evolutionary Genetics Analysis (MEGA) Software Version 5.0 [10] using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method.

Results
Genetic diversity of B. tabaci population of Rajendranagar, Telangana
In the present study B. tabaci specimens collected from okra fields of Rajendranagar of Telangana were subjected to molecular characterization to find out the genetic diversity within the whitefly species complex using mitochondrial cytochrome oxidase I (mtCOI) based Bayesian phylogenetic analysis and sequence divergence.

Sequence analysis
The results obtained from the present study revealed the amplification of mtCOI gene fragment using the primers (C1-J-2195 and L2-N-3014) produced B. tabaci specific ~860bp band in all the samples of whitefly (Fig 1). The amplified products of mtCOI gene fragment was eluted and cloned into pGEM-T Easy sequencing vector. Plasmids from selected colonies were sequenced.

Phylogenetic analysis
The mtCOI sequences of B. tabaci samples of Rajendranagar, Telangana were submitted to the NCBI (National Centre for Biotechnology Information) for nucleotide and protein BLAST. Mitochondrial cytochrome oxidase I (mtCOI) gene of this B. tabaci samples collected from Rajendranagar, Telangana were compared with other known sequences and the test samples showed high level of nucleotide and amino acid identity with Asia I and Asia II-1 genetic groups (Table 1). The phylogenetic tree constructed based on UPGMA (Unweighted Pair Group Method with Arithmetic Mean) analysis with 10 mtCOI sequences of Bemisia tabaci samples from Rajendranagar and Bemisia atriplex (GU086362) was utilized as the outgroup (Fig 2). It was noticed that Rajendranagar B. tabaci population clustered with sequences corresponding to Asia I and Asia II-1 genetic groups. Among the 10 B. tabaci samples, five samples viz., RN1, RN2, RN5, RN9, RN10 belonged to Asia I genetic group whereas other five samples clustered in Asia II-1 genetic group.

Discussion
The survey and analyses performed here provided a detailed picture of species composition and diversity within whitefly complex in Rajendranagar, Hyderabad region of Telangana state. The study revealed the presence of Asia II-1 genetic group in Telangana which was not reported during previous studies. The scientific consensus so far has been that only Asia I genetic group is present in Telangana Region. Contrary to that, this study showed the presence of both Asia I and Asia II-1 genetic groups in Telangana region.
Our survey and the data from GenBank showed that Asia I and Asia II-1 genetic groups were widely distributed across Rajendranagar, Telangana. The existing reports available on the distribution of individual cryptic species of B. tabaci in India indicated the presence of five species namely Asia I, Asia II-1, Asia II-3, Asia II-7, Asia II-8 and MEAM I [12, 13]. Asia in general, is home for at least 17 cryptic species of B. tabaci including globally invasive MEAM I and MED [5, 7]. The Presence of Asia I genetic group was also reported by [10] from Karimnagar district of Telangana. They stated that Asia I was present throughout India with their distribution in states of southern (Andhra Pradesh and Tamil Nadu), Western (Gujarat and Maharashtra) and Northern (Delhi and Uttarakhand) parts of India. The presence of Asia I and Asia II-1 across India was reported by [6] with a wide distribution in southern states with occurrence of more than one genetic groups in the same field. But so far there is no report on the presence of Asia II-1 genetic group from the Telangana state.

There are many factors that determine the survival and population build up of whitefly population. The important ones are: fecundity, egg to adult survival, and above all, resistance to insecticides. A major factor behind the displacement of whitefly populations was its varying capacity towards insecticide resistance [3]. A previous study [3] found that Asia II-1 has very high level of insecticide resistance so much so that Asia II-1 is seen as the most efficient vector of cotton leaf curl virus [14].

Table 1: Percent nucleotide sequence identity of mtCOI gene of Bemisia tabaci collected from Rajendranagar, Telangana with other known sequences in GenBank.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Query coverage (%)</th>
<th>% Identity</th>
<th>Genetic group</th>
</tr>
</thead>
<tbody>
<tr>
<td>RN-1</td>
<td>100</td>
<td>99</td>
<td>Asia I</td>
</tr>
<tr>
<td>RN-2</td>
<td>99</td>
<td>99</td>
<td>Asia I</td>
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<tr>
<td>RN-3</td>
<td>99</td>
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<td>Asia II-1</td>
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<td>RN-4</td>
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<td>97</td>
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<tr>
<td>RN-10</td>
<td>98</td>
<td>99</td>
<td>Asia I</td>
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Fig 1: Amplification of mt COI gene fragment using the universal primer (C1-J-2195 and L2-N-3014). L: DNA Ladder; (1-10) Bemisia tabaci samples collected from Rajendranagar, Telangana.

Fig 2: UPGMA (Unweighted Pair Group Method with Arithmetic Mean) phylogenetic tree of the mtCOI gene from Bemisia tabaci samples collected at Rajendranagar, Telangana. Bemisia atriplex (GU086362) was used as the outgroup.
Conclusions
From the present study, it can be concluded that the occurrence of highly insecticide resistant Asia II-1 genetic group might have contributing to the high level of transmission of Okra Yellow Vein Mosaic Virus (OYVMV) and subsequent severe incidence of OYVMV disease during the recent years in Telangana state. The information generated here could be useful for monitoring future patterns of whitfly population diversity, species composition, abundance and displacement. Although the exact reasons for the observed patterns of diversity are not known, the information obtained from the study has the potential to develop efficient management strategies for whitfly and whitfly transmitted OYVMV disease in okra.

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References