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# Identification of Cryptic Species of *Bemisia tabaci* Associated with Tomato in Eastern Dry Zone of Karnataka

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Abstract

The whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: *Aleyrodidae*) is an economically important crop pest globally that costs growers billions of dollars to control. The nucleotide sequences of mitochondrial cytochrome oxidase subunit 1 (*mtCOI*) gene obtained from sample of *B. tabaci* collected from Tippuru Village, Doddaballapura taluk, Bengaluru Rural (BR) districts of Karnataka was analysed. The total DNA was extracted by Chelex 100 method from single adult whitefly and DNA sample was subjected to PCR using specific primers mtCoIF and mtCOIR for amplifying *mtCOI* gene, which resulted in the expected PCR amplicon of 800 bp size corresponding to mitochondrial cytochrome oxidase gene sub unit 1. The nucleotide similarity search in the NCBI database confirmed the presence of *mtCO1* gene and sequences of cryptic species of *B. tabaci* showing maximum identity were retrieved for analysis. The mtCOI gene sequence from *B. tabaci* collected from Doddaballapur showed maximum nucleotide identity of 94.80 per cent with sequences reported from Nepal, which were identified as Asia-II-5 sequences. The findings were well supported by the sequence demarcation graph and phylogenetic analysis. This confirms that whitefly cryptic species collected from Tippuru village, Bengaluru Rural District as *B. tabci* Asia-II-5 group.

*Keywords* : *Bemisia tabaci,* Mitochondrial cytochrome oxidase subunit 1 (*mtCOI*), Chelex, Cryptic species

Tomato (*Solanum lycopersicum* L.) belongs to the family *Solanaceae* is one of the important vegetable crops grown across the world. It is consumed as raw vegetable, as well in the form of cooked and processed foods. Tomato, apart from being rich in vitamins and minerals, used for the extraction of pigment lycopene, is extensively used in the food industry. In India, tomato is grown in an area of 7,74,000 hectares with production of 18.73 million tones and stands second in global production (Anonymous, 2018). Even though, tomato is grown throughout the country, Andhra Pradesh state leading in its area and production (Chauhan *et al*., 2011).

Pests and diseases are the major constraints for the production of tomato in the country. Among these the sucking pests *viz*., thrips, whiteflies and aphids apart from causing direct damage, are playing a crucial role in transmission of plant viruses resulting in devastating effect on several crop plants. Whitefly (*Bemisia tabaci* Gennadius, Hemiptera: Aleyrodidae) is a widely distributed polyphagous pest in tropical and subtropical regions of India. Both adult and nymph suck the cell sap from phloem by secreting honeydew, causing weakening and dryness of plant (Das *et al*., 2017).

*B. tabaci* is the vector of several viruses, of which, begomoviruses are the most numerous and economically most significant (Rajeswari and Reddy, 2014). *Tomato leaf curl virus* (ToLCV) is the most devastating virus infecting tomato crop. ToLCV is transmitted by whitefly (*Bemisia tabaci*) in a persistent circulative manner (Raghavendra *et al*., 2019).

*B. tabaci* is considered as a cryptic species complex (De Barro *et al.,* 2011). Several studies have reported that at least 44 distinct cryptic species of *B. tabaci* are globally distributed (Dinsdale *et al*., 2010; De Barro *et al*., 2011; Chowda-Reddy *et al*., 2012 and Hu *et al*., 2018). The individual cryptic species within the complex differ in their adaptability to hosts,

resistance to chemicals, degree of fecundity and importantly in their ability to transmit the begomoviruses. As the different members of species complex are morphologically indistinguishable, various molecular methods have been applied over the past two decades to delimit the members of *B. tabaci* species complex. In the recent past, the most widely applied method has been based on mitochondrial cytochrome oxidase gene subunit-1 (*mtCO1*). In the presented study, *mtCO1* gene of whitefly, *B tabaci* species was done and cryptic species was identified based on sequence analysis.

## Material and Methods

### Collection of Whitefly Samples

Adult whiteflies (*B. tabaci*) were collected from tomato field located at Tippur Village (13.3499$^0$ N, 77.4576$^0$ E) Doddaballapura taluk, Bangalore Rural District, Karnataka State using hand held aspirator, where tomato is grown extensively and tomato leaf curl disease is a major problem. The collected whiteflies were transferred to 1.5 ml eppendorf tube containing 70 per cent alcohol. The eppendorf tube was sealed properly using parafilm and sample was labeled by giving the details of sample number, location and date. Sample was brought to the laboratory and store at 4 $^0$C until further processing.

### Extraction of DNA, PCR Amplification of *mtCOI* gene and its Sequencing

The total DNA from whitefly, *B. tabaci* sample was extracted by modified Chelex100 by Rua *et al*. (2006). Single whiteflies were removed from collection tube containing 70 per cent ethanol and placed on a strip of parafilm by using camel hair brush to facilitate the evaporation of ethanol. Later the flies were transferred to Petridish and washed with sodium hypochlorite (0.1%) followed by sterile distilled water (SDW), twice. Single whitefly was transferred to individual 1.5 ml microcentrifuge tube. Each whitefly was homogenized in 100µl TE buffer solution containing 5 per cent Chelex 100 raisin and 300µg Proteinase K. Homogenised sample was incubated at 60 $^0$C for 3 hours followed by protein denaturation at 96 $^0$C for 10 minutes. Homogenised sample was then centrifuged at 13,000 rpm for 10 minutes and upper aqueous supernatant continuing DNA was pipetted out into a fresh tube and stored at -20$^0$ C.

### Polymerase Chain Reaction (PCR) Amplification of *mtCOI* gene of *B. tabaci* was done

The DNA sample was subjected to PCR using universal random primers, mtCoIF and mtCOIR specific to the *mtCOI* gene of *B. tabaci*. Total volume of the reaction mixture was 25µL, composed of deionized nuclease free water (14.5µL), 10 x PCR buffer (2.5µL), 25 mM MgCl2 (2.0µL), 2.5 mM dNTP mixture (2.0µL), 1.2µL (10mM) of each primer, 0.3µL Taq polymerase (1000 units), 1.3µL Template DNA (100ng). The details of the primer sequences, cyclic conditions and expected amplicon size are given in the Table 1. Five microliters of PCR product was electrophoresed on 1 per cent agarose gel, ethidium bromide stained and visualized under gel

Table 1

Details of universal primers used for amplification of *mtCOI* gene of *B. tabaci* and PCR cycle parameters

| Primers details | Primer sequences (5' to 3') | PCR conditions | Product size (bp) |
|---|---|---|---|
| Forward | | Initial denaturation : 94 ºC for 5 min | 800bp |
| | TTGATTTTTTGG | Denaturation : 94 ºC for 45 sec | |
| Reverse | TCATCCAGAAGT | Annealing : 55 ºC for 1 min | |
| | | Extension : 72 ºC for 1 min | |
| | TCCAATGCACTA | Final extension : 72 ºC for10 min | |
| | ATCTGCCATATTA | | |

*Note*: 35 cycles were followed

documentation system. Remaining PCR product of the sample amplification was sent for sequencing at Medauxin Pvt. Ltd., Bangalore.

**Sequence Analysis of *B. Tabaci* Doddaballapur Isolate**

The obtained forward and reverse sequences of *mtCOI* were used for generating consensus sequences using Bioedit Sequence Alignment Editor (version 5.0.9) (Hall, 1999). The similarity search for *mtCOI* gene nucleotide sequences was done at National Center for Biotechnology Information (NCBI) database using Basic Local Alignment Search Tool (BLASTn) (https://blast.ncbi.nlm. nih.gov/Blast.cgi). The sequences with maximum similarity were retrieved from the GenBank. Sequence Demarcation Tool version 1.2 (SDTv1.2) was used to calculate pairwise per cent identity between *B. tabasi* DB isolate and the retrieved sequences. With the aid of MEGA x software (Kumar *et al.,* 2016) phylogeny was generated using the neighbor joining method with 1000 bootstrap replications.

## Results and Discussion

**Whitefly, *B. tabaci mtCOI* Gene Amplification and Sequencing**

The isolated genomic DNA of *B. tabaci* was subjected to PCR for the amplification of *mtCOI* gene using specific primers. The PCR amplification resulted in an expected amplification size of 800bp (Fig.1). Several workers used these primers for amplification of *mtCOI* gene in the whitefly, *B. tabaci* diversity analysis (Dinsdale *et al*., 2010, Himler *et al*., 2011 and Ashwathappa *et al.,* 2020). The obtained nucleotide (nt) sequence was compared with the other *B. tabaci* nucleotide sequences present in the NCBI database. The nt sequence similarity search confirmed the presence of *mtCOI* gene in our *B. tabaci* Doddaballapur isolate. The results obtained were well supported by the earlier reports (Raghavendra, 2018).

The amplified *mtCOI* gene sequences obtained from *B. tabaci* sample was subjected to percent nt similarity check at the NCBI by using the BLASTn. Selected sequences *mtCOI* gene of different cryptic species of

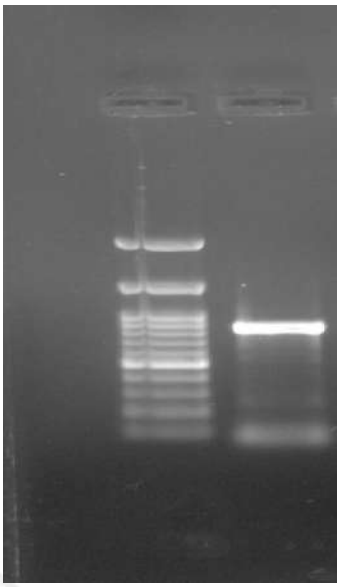

Fig. 1. PCR based detection of cryptic species of *B. tabaci* (Asia-II-5) by PCR using *mtCOI* gene specific primers

*B. tabaci* showing maximum similarity with the current sequence were retrieved and compared. Comparison revealed that *mtCOI* gene sequence of *B. tabaci* sample in the present is having 94.80 per cent identity with the sequences (MN840128, MN840129, MN840130 and 840111) reported from Nepal (Table 2). Hence, based on this *B tabaci* collected for identification is Asia II- 5 cryptic species. Further, the *B. tabaci* Doddaballapur isolate was compared with 20 other *B. tabaci mtCOI* nt sequences retrieved from the NCBI database. This result was well supported by the sequence demarcation graph generated using SDTv1.2 (Fig. 2). To find the ancestral relationship, the phylogenetic analysis was carried out. The phylogenetic tree showed that the *mtCOI* gene amplified from the whitefly, *B. tabaci* collected in the current study is closely clustering with *B. tabaci* isolates belonging to the Asia-II-5 group (Fig. 3). This analysis further confirms that the *B. tabaci* collected from Doddaballapur belongs to the Asia-II-5 group of whitefly cryptic species.

The whitefly is the only known vector so far for transmitting the begomoviruses. The transmission efficiency of the virus depends on the type of cryptic whitefly species population present in the crop field (Venkataravanappa *et al*., 2017). Similar attempt was

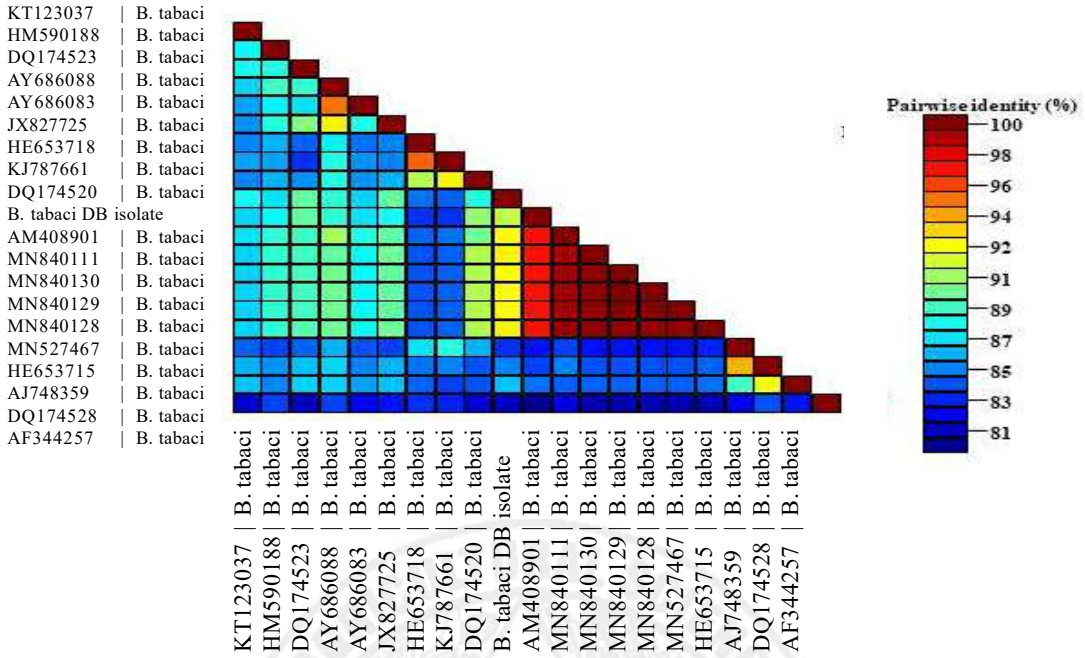


Fig. 2: Graphical representation of percentage pairwise genome scores and nucleotide identity plot of *B. tabaci* cryptic species collected from tomato plot compared with other reference sequences - prepared using Sequence Demarcation Tool (SDTv1.0)

TABLE 2

Pairwise nucleotide identity of *B. tabaci* cryptic species collected from tomato plot compared with other reference sequences

| Accession number | Organism | Country | Per cent nucleotide identity of *B. tabaci* Doddaballapura isolate with others |
|---|---|---|---|
| MN527467 | *Bemisia tabaci* | India | 93.10 |
| MN840130 | *Bemisia tabaci* | Nepal | 94.80 |
| MN840129 | *Bemisia tabaci* | Nepal | 94.80 |
| MN840128 | *Bemisia tabaci* | Nepal | 94.80 |
| MN840111 | *Bemisia tabaci* | Nepal | 94.80 |
| HE653715 | *Bemisia tabaci* | Netherland | 81.00 |
| AJ748359 | *Bemisia tabaci* | India | 81.40 |
| DQ174528 | *Bemisia tabaci* | Taiwan | 77.60 |
| KT123037 | *Bemisia tabaci* | China | 82.60 |
| HM590188 | *Bemisia tabaci* | India | 85.80 |
| DQ174523 | *Bemisia tabaci* | China | 82.00 |
| AM408901 | *Bemisia tabaci* | India | 83.70 |
| DQ174520 | *Bemisia tabaci* | Taiwan | 83.50 |
| AY686088 | *Bemisia tabaci* | China | 83.70 |
| JX827725 | *Bemisia tabaci* | India | 81.10 |
| AY686083 | *Bemisia tabaci* | China | 82.10 |
| AJ550177 | *Bemisia tabaci* | Reunion | 81.80 |
| HE653718 | *Bemisia tabaci* | Netherland | 81.90 |
| KJ787661 | *Bemisia tabaci* | India | 88.30 |
| AF344257 | *Bemisia tabaci* | Cameroon | 78.10 |

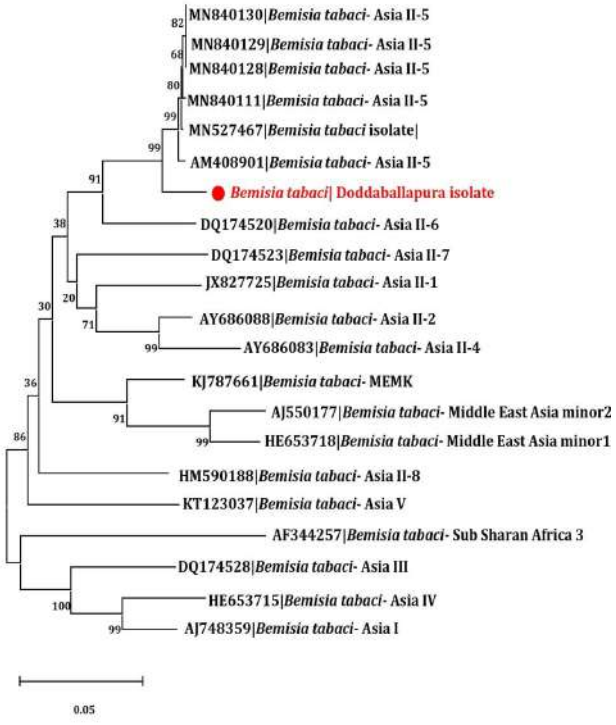


Fig. 3: Phylogenetic tree showing the relationship of cryptic species of *B. tabaci* (Asia-II-5) sequence collected in this study

made to identify the cryptic whitefly species (Asia II-5 group population) associated with Tomato crop (Ashwathappa *et al*., 2020).

The present findings are supported by Chowda Reddy *et al*., 2012 who reported that Asia I, Asia II 5, Asia II 7, Asia II 8 and MEAM 1 from India by deploying RAPD - PCR and *mtCOI* gene sequencing data obtained from whitefly samples collected from 31 regions of India. Similarly, MEAM 1 along with Asia I, Asia I-India, Asia II-1, Asia II-5, Asia II-7, Asia II-8 and Asia II -11 cryptic species of whitefly were reported, based on *mtCOI* gene sequences (Ellango *et al*., 2015 and Prasanna *et al*., 2015).

The present study was also supported by Acharya *et al*., 2020 who identified the presence of three cryptic species, Asia I, Asia II 1 and Asia II 5 with high interspecific but low intraspecific variations. Among the three cryptic species, Asia II 5 was the most prevalent in Nepal, constituting 64.47 per cent of all the sequenced samples.

Roopa *et al.* (2015) analyzed *mtCOI* gene sequences from 71 samples of *B. tabaci* to determine the prevalence of genetic groups on various host plants in India. Results revealed the prevalence of a new group called Middle East Asia Minor-K, which is genetically close to MEAM-1 in addition to the four previously existing genetic groups, namely Asia-I, Asia-II-7, Asia-II-8, and Middle East Asia Minor-1 (MEAM-1). Mitochondrial cytochrome oxidase I gene sequences and randomly amplified polymorphic DNA (RAPD) polymorphisms, generated using the primer OpB11, were also found useful for detecting *B. tabaci* biotypes (Shankarappa *et al*., 2007).

There are many factors that determine the successful survival and population build-up of whitefly population such as fecundity, egg to adult survival, and most importantly resistance to insecticide and susceptibility to parasitoids. The effect of differential capacity to resist insecticide is well known to contribute to predominance and displacement of specific whitefly populations (Crowder *et al.*, 2010).

This current study unifies our knowledge of the species composition of *B. tabaci* from Tippuru Village, Doddaballapura taluk, Bengaluru Rural district, Karnataka. The data from PCR analysis and the mtCOI based phylogeny and Pairwise nucleotide identity analysis supported the existence of Asia II-5 cryptic species in Tippuru village, Doddaballapura taluk, Bengaluru Rural district, Karnataka. The information generated here could be useful for monitoring future patterns of whitefly population diversity, abundance and displacement. It would be interesting to conduct more intensive surveys in this region to evaluate whether the species composition of *B. tabaci* cryptic species is higher than that described so far.

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