



IDENTIFICATION OF *BEMISIA TABACI* (GENNADIUS) INFESTING MULBERRY USING MITOCHONDRIAL COENZYME 1 GENE SEQUENCE

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ABSTRACT: *Bemisia tabaci* is the vector of *Mulberry mosaic disease* (MMD) reported from Kerala, India. *Bemisia tabaci* collected from mulberry plants were maintained on two different host plants, cassava and *Nicotiana tabacum* for developing pure culture. After completion of 20 generations the mtCO1 gene of the red eye nymphs of *B. tabaci* collected from this population were analyzed and found to have 11% divergence. The population collected from cassava showed similarity to AsiaII_7 group known from India, China, Indonesia and Taiwan while that from *N. tabacum* matched with AsiaII_8 group reported from India only. Mulberry is the common host of two species of *B. tabaci* belonging to two groups. The host preference of these species resulted in the development of only the specific species in each host plant in the pure culture. The presence of *B. tabaci* belonging to two different groups on mulberry can lead to the formation of new recombinants of virus, as the other host plants of the fly belonging to different families are infected with specific virus and it will be transferred to mulberry while feeding on it.

Key words: *Bemisia tabaci*; *Morus alba*; genotypes; mtCO1

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INTRODUCTION

India is the second largest producer of silk in the world. The productivity in terms of mulberry silk has increased to 93.38/kg/ha/year from 40kg/ha/year compared to the yesteryear. Introduction of high yielding mulberry varieties, improved silkworm breeds, new management practices and processing machinery are the contributing factors for the above achievement. Mulberry (*Morus alba* L) is the only food plant of *Bombyx mori* L. Fungal, bacterial and viral diseases are reported from mulberry. Remarks about *Mulberry Mosaic Dwarf Disease* (MMDD) were found in Sheng agriculture book published in the end of Ming dynasty, in 40s of 17th century [1] and from India it has been reported from different locations from time to time [2,3,4,5,6,7]. Symptoms typical to viral diseases are found in Victory1 (V1) a high yielding variety released by CSR&TI, Mysuru [6]. The mulberry mosaic disease reported on V1 variety expressed typical leaf symptoms of plants infected with Geminivirus viz., yellow-green mosaic, curling, and malformation and size reduction of leaves [7]. The mulberry mosaic disease on its sequence analysis of coat protein and movement protein revealed its close relationship to *Indian cassava mosaic virus* (ICMV) and *Sri Lankan cassava mosaic virus* (SLCMV), the two common cassava mosaic viruses reported from India, belonging to the genera Begomovirus of family Geminiviridae. Begomoviruses, the largest and most economically significant group of plant viruses [8, 9] are transmitted exclusively by *B. tabaci* in a circulative, persistent and non propagative manner [10, 11, and 12].

It is the largest genus of the family of single stranded DNA plant viruses, which consists of more than 180 species and several unassigned isolates [9]. Earlier reports of *Bemisia tabaci* on mulberry are available [13, 14]. It has been reported as the vector of yellow net vein disease of mulberry [15, 16]. From India host specific whitefly biotypes from cassava and sweet potato was reported and named as cassava strain and sweet potato strain [17]. The sweet potato reared population did not breed on cassava, and the cassava strain failed to develop on sweet potato. Egg plant and tobacco were the common hosts for both biotypes. Two genetically divergent *B. tabaci* populations viz., north and south group were reported from Karnataka [18]. In India, occurrence of B-biotype was also reported in Kolar region of South India [19]. It was opined that within a narrow geographical region there exists variation based on host plants being utilized by the whitefly population (20). Host plant specific genotypes in *B. tabaci* reported (21) showed a high level of genetic relatedness (72-85%) based on the dendrogram up on the RAPD profiles and based on this lineage three groups has been suggested. Even though *B. tabaci* had been reported as a sucking pest of mulberry so far no attempt has been made to identify genotypes of the *B. tabaci* on mulberry. In this study an analysis using mtCO1 gene sequences of the *B. tabaci* populations on mulberry were done by maintaining the pure culture of the *B. tabaci* population collected from mulberry on cassava (*Manihot esculenta*) and *Nicotiana tabacum* seedlings.

MATERIALS AND METHODS

Red eye nymphs, the pupal stage of *B. tabaci* were collected from the mulberry plants maintained in glass house. They were kept in glass Petri plates of 20 cm diameter and the emerged flies five pair each was released on seedlings of cassava and *N. tabacum* var. Delcrest kept in separate insect proof cages of 70cm (height) by 42cm by 42cm covered with organdy cloth on three sides and transparent plastic sheet on top and the fourth side. The old plants were replaced introducing insect free saplings periodically. Red eye nymphs were collected from this pure culture after completion of 20 generations.

DNA isolation and sequencing

The red eye nymphs collected from the above colonies were fixed in 100% ethanol. From the DNA isolated [22] mtCO1 region was amplified using Primers C1-J-2195 in combination with TL2-N-3014 [23]. The PCR done [24] and the amplified products were sequenced by Macrogen Inc. (Seoul, Korea).

In silico analysis

Sequences were edited using the BioEdit software 7.0 [34]. The nucleotide sequences were compared with those in the NCBI database using the Basic Local Alignment Search Tool (BLAST, <http://www.ncbi.nlm.nih.gov/BLAST>). The sequences obtained were aligned using sequences from NCBI which showed similarity to them using Clustal W multiple alignment programme of BioEdit software. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model [35]. Estimates of Evolutionary Divergence between Sequences were conducted using the Tajima-Nei model [36] and MEGA6 [37]. Compared the two unknown sequences GU353180 (*N. tabacum*) and GU353179 (cassava) to the sequences in the publicly available CSIRO dataset <https://data.csiro.au/dap/landingpage?pid=csiro:6002>.

RESULTS

The BLAST result of the sequences of the nymphs collected from the pure culture maintained on cassava and *N. tabacum* showed that they are genetically different. They have 11% divergence. The population collected from cassava showed 99.5% match with the consensus sequences of AsiaII_7 known from India, China, Indonesia and Taiwan while that from *N. tabacum* matches with AsiaII_8 from India only. AsiaII_7 has been recorded from *Parthenium hysterophorus*, *Codiaeum variegatum*, soybean, watermelon, cotton, Ipomoea sp., *Gossypium hirsutus*, *Euphorbia pulcherrima* (poinsettia), *Capsicum annum*, sunflower [GQ139492; DQ174523; AY686064; AJ748372; AJ748375; AJ748378; DQ116650; DQ116660; DQ116661; DQ116662; AM408899; DQ174522; DQ174521; DQ174524; AY686075] and now from mulberry. These accessions belong to biotype Cv.

The population from *N. tabacum* showed 100% similarity to accessions. AsiaII_8 which has been recorded from *Acanthospermum hispidium*, *Ageratum conyzoides*, cluster bean, potato, tomato, *Solanum melongena*, cotton, okra [AJ748357; AJ748358; AJ748362; AJ748374; HM590181; AM408898; AM040593; HM590182; HM590184; HM590185; HM590188] and now from mulberry.

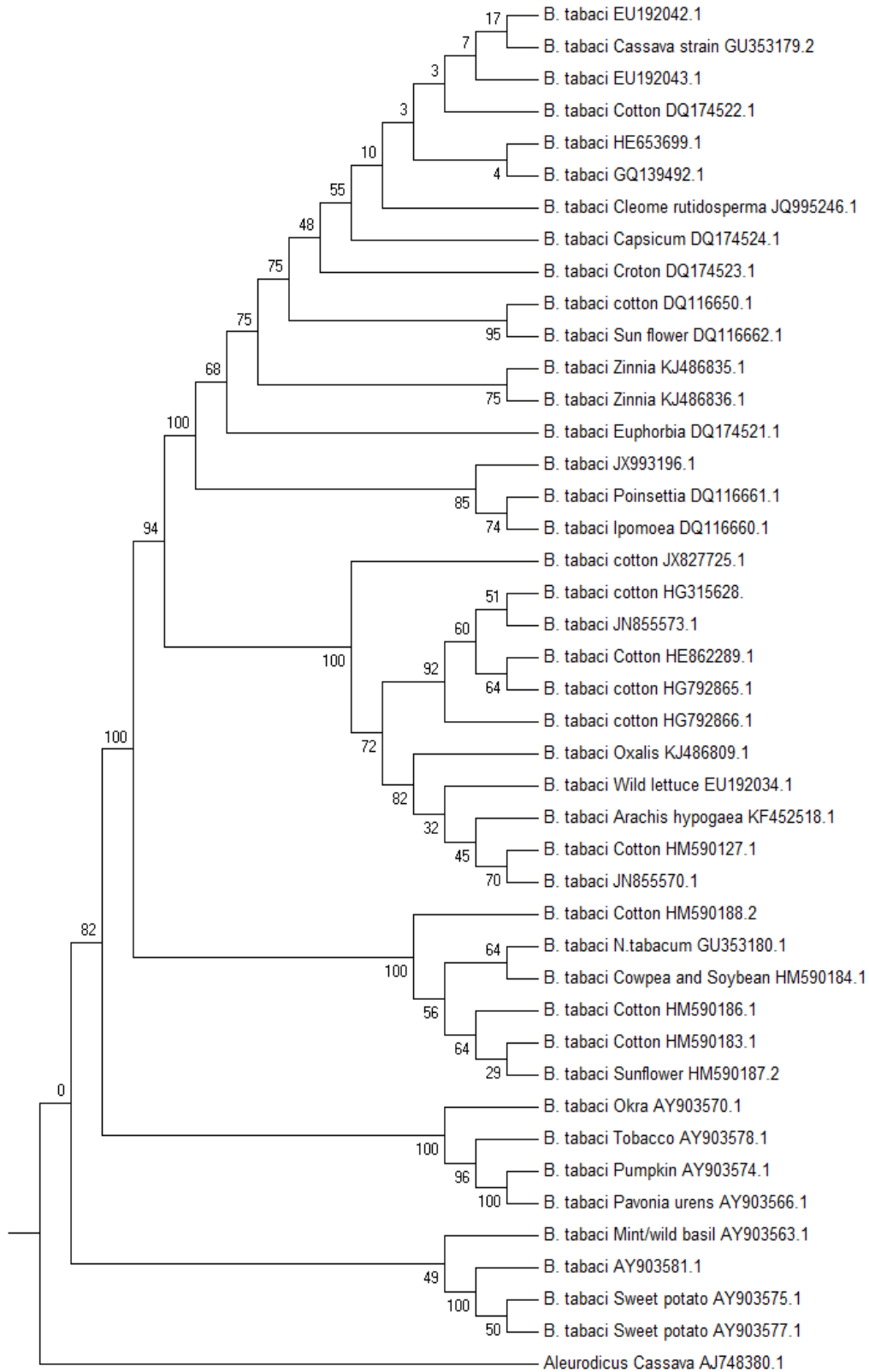


Fig.1. Relationship dendrogram of the mtCO1 nucleotide of whiteflies from the 20 generation culture on *N. tabacum* and cassava with whiteflies from different host plants.

Table.1 Details of the accessions used for making dendrogram with mtCO1 nucleotide sequence of cassava strain.

Isolate Name	Accession number	Host plant	Percentage of similarity	Country
<i>Bemisia tabaci</i> isolate11	EU192042.1	Not known	99%	China
<i>Bemisia tabaci</i> isolate 12	EU192043.1	Not known	99%	China
<i>Bemisia tabaci</i>	DQ174522.1	<i>Gossipium hirsutum</i>	99%	China
<i>Bemisia tabaci</i>	HE653699.1	Tomato	99%	Netherlands
<i>Bemisia tabaci</i>	GQ139492.1	Not known	99%	China
<i>Bemisia tabaci</i>	DQ174521.1	<i>Euphorbia pulcherrima</i>	99%	Taiwan
<i>Bemisia tabaci</i>	JQ995246.1	<i>Cleome rutidopserma</i>	99%	India
<i>Bemisia tabaci</i>	DQ174524.1	<i>Capsicum annum</i>	99%	Indonesia
<i>Bemisia tabaci</i>	DQ174523.1	<i>Codiaeum variegatum</i>	99%	China
<i>Bemisia tabaci</i>	DQ116650.1	<i>Gossipium hirsutum</i>	99%	India
<i>Bemisia tabaci</i>	DQ116662.1	Sunflower	99%	India
<i>Bemisia tabaci</i>	KJ486835.1	<i>Zinnia</i>	99%	Pakistan
<i>Bemisia tabaci</i>	KJ486836.1	<i>Zinnia</i>	99%	Pakistan
<i>Bemisia tabaci</i>	DQ174521.1	<i>Euphorbia pulcherima</i>	99%	Taiwan
<i>Bemisia tabaci</i>	JX993196.1	Not known	99%	India
<i>Bemisia tabaci</i>	DQ116661.1	<i>Poinsettia</i>	99%	India
<i>Bemisia tabaci</i>	DQ116660.1	<i>Ipomoea</i>	99%	India
<i>Bemisia tabaci</i>	JX827725.1	<i>Gossipium hirsutum</i>	92%	India

Table.2 : Details of the accessions used for making dendrogram with mtCO1 nucleotide sequence of *N.tabacum* strain

Isolate Name	Accession number	Country	Host plant	Percentage of similarity
<i>Bemisia tabaci</i>	KR020542.1	India	<i>Abelmoschus esculentus</i>	100%
<i>Bemisia tabaci</i>	KR020539.1	India	<i>Vigna radiata</i>	100%
<i>Bemisia tabaci</i>	KR020539.1	India	<i>Vigna mungo</i>	100%
<i>Bemisia tabaci</i>	KJ663486.1	India	<i>Vigna mungo</i>	100%
<i>Bemisia tabaci</i>	KJ663485.1	India	<i>Solanum lycopersicum</i>	100%
<i>Bemisia tabaci</i>	KJ663483.1	India	<i>Solanum torvum</i>	100%
<i>Bemisia tabaci</i>	KJ663477.1	India	<i>Vigna mungo</i>	100%
<i>Bemisia tabaci</i>	KJ663476.1	India	<i>Vigna mungo</i>	100%
<i>Bemisia tabaci</i>	KJ663454.1.1	India	<i>Vigna mungo</i>	100%
<i>Bemisia tabaci</i>	KJ663453.1	India	<i>Vigna mungo</i>	100%
<i>Bemisia tabaci</i>	KJ663452.1	India	<i>Solanum lycopersicum</i>	100%
<i>Bemisia tabaci</i>	KJ663451.1	India	<i>Vigna radiata</i>	100%
<i>Bemisia tabaci</i>	KJ663450.1	India	<i>Solanum melongena</i>	100%
<i>Bemisia tabaci</i>	JX993198.1	India	Not Known	100%
<i>Bemisia tabaci</i>	KJ523177.1	India	Not Known	100%
<i>Bemisia tabaci</i>	GQ281725.1	India	<i>Gossypium hirsutum</i>	100%
<i>Bemisia tabaci</i>	AF418664.1	India	<i>Euphorbia geniculata</i>	100%
<i>Bemisia tabaci</i>	AJ748357.1	India	<i>Acanthospermum hispidum</i>	100%
<i>Bemisia tabaci</i>	HM590187.2	India	Sunflower	99%
<i>Bemisia tabaci</i>	HM590184.1	India	Cowpea and soybean	99%
<i>Bemisia tabaci</i>	HM590188.2	India	Cotton	99%

DISCUSSION

Bemisia tabaci collected from mulberry plants on analysis of their mtCO1 gene showed 11% divergence and the colony maintained on cassava has close relations to populations from China, Taiwan, Indonesia and India. The *N. tabacum* population is close to populations from South India. The culture on cassava is 99.5% similar to populations belonging to AsiaII_7 group of Cv biotype and had only 89% similarity to that of Asia II_8 group whereas the *N. tabacum* strain is 100% similar to populations belonging to AsiaII_8 group and 89% similar to that of Asia II_7 group of biotype Cv. Field samples of adult *B. tabaci* on analysis gave the same result, flies collected from mulberry plants only 200m apart had 10% divergence which supports the result obtained from the pure culture. Of these two, flies cultured on cassava was 98-100% similar to that collected from cassava and irrespective of location the flies collected from cassava had 100% match [26]. According to Dinsdale *et al.* [24] and De Barro *et al.* [27] samples having more than 11% divergence belongs to different groups and those with more than 3.5% divergence belongs to different species. De Barro *et al.* [27] discarded the use of the term biotype and according to the DNA sequence divided *B. tabaci* into 11 groups with 11% divergence and 24 species with 3.5% divergence. The tips to root analysis of Boykin *et al.* [25] observed more groups in the clades than reported by De Barro *et al.* [27]. Definition of *B. tabaci* as a species complex having numerous cryptic species is found to be true in this study. From the result obtained after analysing the mtCO1 it is evident that mulberry acts as the host of *B. tabaci* belonging to different groups. The findings of Dinsdale *et al.* [24]. and De Barro *et al.* [27] were supported by the available data on reproductive isolation [28]. Lepidopteran divergence values 3% corresponded with 196 of 200 species recognized from previous morphological studies [29]. Abdullahi *et al.* [30] established that whitefly populations associated with cassava were restricted to it, whereas *B. tabaci* from other hosts were polyphagous but did not colonize on cassava. De Barro [31] has objected this race concept [30, 32, and 33] stating that the conclusion made by those who advocated it was only one sided as the host preference study was not conducted on other plants to see whether the flies can adopt on them. Lisha *et al.* [17] has noted *N. tabacum* and egg plant as the common host of two biotypes, cassava and sweet potato strain, identified by enzyme analysis from the same study area of present investigation. Individuals from the sweet potato reared population did not breed on cassava, and the cassava strain reared individuals failed to develop on sweet potato.. *Bemisia tabaci* from cassava in Africa is considered as a distinct group. Phylogenetic analysis of *B. tabaci* mtCO1 from the African continent has revealed five major cassava-associated haplotypes (38). Two biotypes were identified by Burban *et al.* (33) from cassava and eggplant, the latter is polyphagous, but did not infest cassava. The rejection of main host plants of other biotype was demonstrated using two biotypes (33, 39, and 40). They suggested that this discriminant hosts can therefore serve as a useful tool in the behavioural characterisation and screening of populations of biotypes, like the squash silver leaf bioassay, which is widely used to detect the presence of the B biotype (41, 42,43).

CONCLUSION

Mulberry hosts *B. tabaci* belonging to two different groups AsiaII_7 and AsiaII_8. By hosting *B. tabaci* belonging to two different groups it can act as a reservoir of various viruses belonging to families of the preferred hosts of each group of *B. tabaci*. The transfer of viruses belonging to different genus to mulberry may result in the development of more recombinants. In India mulberry is being cultivated in large scale for silkworm rearing and it is also used as a fruit tree, fodder and hedge plant. Biology of the different biotypes on mulberry and competition or co-living if any has to be studied in detail.

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