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RESEARCH ARTICLE

Occurrence of *Bemisia tabaci* Asia 1 in Association with BYVMV in Okra

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ABSTRACT

Bhendi Yellow Vein Mosaic Virus (BYVMV) incidence caused by white fly is the main bottleneck for cultivation of the okra. The present investigation was carried out in three major okra-growing districts of Tamil Nadu viz., Coimbatore, Dharmapuri and Dindugul on whitefly incidence and occurence. A field survey on these districts revealed that the mean whitefly population of 1.82 per plant was observed while, the incidence of BYVMV in the Coimbatore district was 13 per cent. The least mean population was observed in the Dharmapuri district with a mean of 0.48 whiteflies per plant and BYVMV incidence of 15.75 %. In order to gain insight into whitefly genotypes occurring on Okra (Abelmoschus esculentus L. (Moench), whitefly samples were collected from 8 locations of Tamil Nadu, and their mitochondrial cytochrome oxidase subunit I (mtCOI) gene was molecularly characterized for species identification. sequences results revealed that the whitefly belongs to Asia I genotype. Thus, the present study confirmed the presence of Asia 1 genotype in B. tabaci throughout Tamil Nadu okra growing regions.

Keywords: *Bemisia tabaci*; *Bhendi Yellow Vein Mosaic Virus;mtCOI; Okra*

INTRODUCTION

Okra, *Abelmoschus esculentus* L. (Moench), (Family: Malvaceae) is widely grown in tropical and sub-tropical regions of the world. It is an important vegetable component in the human diet due to its dietary fibers and is rich in magnesium folate, antioxidants, potassium, vitamins C, K1 and A (Hughes, 2008).

The whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is the most devastating insect pest in agricultural crops worldwide (Brown *et al.,* 1995, de Barro *et al.,* 2000). It was first collected and described as *Aleyrodes tabaci* (Gennadius) from tobacco, *Nicotiana tabacum* L., in Greece in 1889. It was subsequently renamed (Russell, 1957) as *B. tabaci* and found across the globe in United States, Africa, Middle East, the Orient, Russia, China, Southeast Asia, and South America (Brown *et al.,* 1995). Its geographical diversity and broad host range gave rise to several common names associated with host plants such as sweet potato whitefly, cotton whitefly, *etc.* Different populations of *B. tabaci* are morphologically undefined but display distinctive biological, physiological, and genetic variation, and thus are deemed a cryptic species complex (Boykin *et al.,* 2007, 2012; de Barro *et al.,* 2011; Dinsdale *et al.,* 2010; Tay *et al.,* 2012). The *B. tabaci* complex consists of cryptic species that need to be separated and distinguished. As these cryptic species are morphologically indistinguishable, various molecular markers have been utilized such as RAPD PCR (Gawel and Bartlett, 1993, de Barro and Driver, 1997), AFLP (Cervera *et al.,* 2000), mitochondrial cytochrome oxidase gene subunit I (*mtCOI*) (Frohlich *et al.,* 1999, Brown *et al.* 2000) and the ribosomal ITS1 nucleotide sequence (de Barro *et al.,* 2000). The most widely accepted method is differentiation on the basis of nucleotide sequence of *mtCOI*. Using *mtCOI*-based Bayesian phylogenetic analysis, Dinsdale *et al.* (2010) and de Barro *et al.* (2011) proposed a speciation framework keeping 3.5% pairwise divergence as threshold. Based on these criteria, recently 42 putative species and 12 major genetic groups have been separated at global level (Kanakala and Ghanim, 2019). Differentiation of cryptic species on the basis of mating behavior, insecticide resistance, oviposition and transmission characteristics was examined. In the present study, the genetic affiliation of *B. tabaci* populations used in

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mtCOI analysis (de Barro *et al.,* 2011) and incidence of *B.tabaci* and BYVMV infestations occurring on bhendi in Tamil Nadu, India were studied.

MATERIAL AND METHODS

The field survey was conducted in three major okra growing districts of Tamil Nadu *viz.,* Dharmapuri, Coimbatore and Dindigul and the incidence of whitefly and Bhendi Yellow Vein Mosaic Virus (BYVMV) were randomly observed in five different locations of each district during 2018 to 2019. Adults of *B. tabaci* were counted on three leaves per bhendi plant, one from top, middle and bottom from ten randomly selected plants per field leaving the border rows . Population count was taken from early morning hours and expressed as number per plants. The location of sample collection and genotypic details are given in table.1. The total number of plants and number of plants infected with BYVMV were calculated from fifty randomly selected plants at the flowering stage leaving the outer two rows on all the four sides in each field and expressed as per cent disease incidence (Venkataravanappa *et al.,* 2012).

$$\text{Per cent incidence of YVMV (\%)} = \frac{\text{Number of YVMV infected plants}}{\text{Total number of plants observed}} \times 100$$

The experiment was replicated three times. The incidence of BYVMV was observed based on characteristic symptoms *viz.,* various levels of chlorosis, yellowing of veins and veinlets, smaller leaves, fewer and smaller fruits and stunting.

Adults of *B. tabaci* were collected from distinct locations in three districts using hand held aspirator for genetic identification. At each location, individual insect samples were collected from okra in separate 1.5 mL Eppendorf tubes containing 70% ethanol and stored in a freezer at - 80^{0}C, until used.

DNA isolation

Genomic DNA was isolated from individual adult whitefly using Hot SHOT method according to Montero-pau *et al.* (2008). Individual insect sample was homogenized with 50 µL of alkaline lysis buffer (125 µL of NaOH, 20 µL of Na_2EDTA (pH 8) and 50 mL of ddH_2O) and transferred to Eppendorf tubes and incubated at 95 °C for 30 min in the water bath and allowed to cool down at 4 ^{0}C. Then 50 µL of neutralizing solution was added (315 mg of Tris-HCL and 50 mL of ddH_2O). The substances were spun and vortexed for 5 s and stored at -20 °C for further analysis.

mtCOI subunit I amplification and sequence analysis

The genomic DNA of whitefly samples collected from 8 locations were confirmed for the presence of *mtCOI* gene using LCO 1490 forward primer 5' GGTCAACAAATCATAAAGATATTGG 3' and HCO 2198 reverse primer 5' TAAACTTCAGGGTGACCAAAAAATCA 3' (Folmer *et al.,* 1994). The PCR reaction mix consisted of 5µL of template DNA (approximately 50 ng), 10.5 µL of sterile distilled water, 2.5 µL of dNTPs, 2.5 µL of PCR buffer, 1.0 µL of $MgCl_2$, 1.5 µL of each forward and reverse primer, 0.5 µL of *Taq* polymerase. PCR was performed with initial denaturation at 94 °C for 2 min, followed by 35 cycles each consisting of denaturation for 1 min at 94 °C, annealing for 1 min at 52 °C with extension for 1 min at 72 °C, followed by final extension for 10 min at 72 °C. The PCR products were eluted and sequenced in Agrigenome Labs Pvt. Ltd., Cochin, Kerala. *mtCOI* gene sequence corresponding to 34 different genetic groups of *B. tabaci* were downloaded from the National Center for Biotechnology Information (NCBI) GenBank (https://www.ncbi.nlm.nih.gov/Blast.cgi). Sequence alignment was performed employing MUSCLE implemented in Seaview (Thompson *et al.,* 1994). The tree was generated by neighbour joining method employing MEGA 7 software (Saitou and Nei, 1987). Genetic divergence was calculated employing MEGA 7 using ClustalW (Kumar *et al.,* 2016). The *mtCOI* DNA sequences generated in the study were submitted to NCBI database.

RESULTS AND DISCUSSION

Incidence of B. tabaci and yellow vein mosaic disease

Among the three districts surveyed the highest mean whitefly population was recorded in Madampatti (9.53) followed by Natham (2.53), Korimedu (2.03) and Arasampalayam (2.03) and the lowest whitefly population of (0.20) was recorded at Madampatti of Coimbatore district. Highest YVMD symptoms (0.86 %) were registered in Coimbatore district, Madampatti, followed by Anjehalli (0.60 %), Natham (0.37 %), Eranahalli (0.35 %) and the lowest (0.05 %) at Nallakulam of Dindigul district (Table 1).

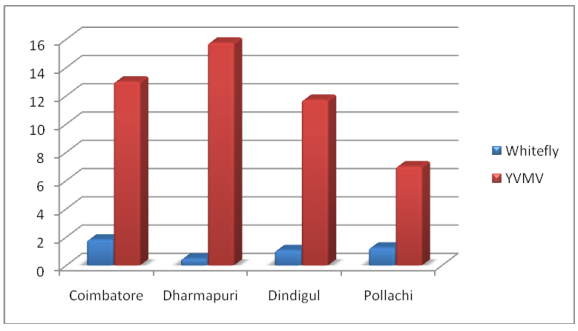

Figure 1. Incidence of whitefly and Bhendi yellow vein mosaic virus (YVMV) disease from four major areas of Tamil Nadu (2018-2019)

Table 1. Incidence of whitefly and Bhendi Yellow Vein mosaic virus (BYVMV) disease in bhendi in Tamil Nadu (2018-2019)

S.No	District	Farmer	Location	Bhendi Variety/ hybrid	Age of the crop (DAS)	YVMV incidence No/50 plants	No of whiteflies/ 10 plants	Location
						Mean	Mean	
1	Coimbatore	Nagarathinam S/o Murugasen	Puthur	Shakthi, Nunhems	60	0	0	10.97°N, 76.83°E
		Arumugam S/o Rajendiran	Thondamuthur	Samrad, Nunhems	75	7	0	10.98°N, 76.84°E
		Murugan S/o Venugopal	Thondamuthur	3312, Syngenta	45	0	0.53	10.98°N, 76.84°E
		Balakrishnan S/ oPalaniyappan	Narasipuram	Radhika, UPL	75	0	0	10.98°N, 76.77°E
		Jeyaraman S/o Krishnamoorthy	Madampatti(1)	Novo, Vairam	90	16	0.20	10.96°N, 76.85°E
		Selvaraj S/o Karrupaswamy	Madampatti(2)	Venus plus, UPL	70	86	9.53	10.96°N, 76.85°E
		Mani S/o Padmanaban	Madampatti(3)	102, Syngenta	90	8	0	10.96°N, 76.85°E
2	Dharmapuri	Parameswaran S/o Velusamy	Perur	Radhika, UPL	45	0	0.67	10.97°N, 76.91°E
		Marimuthu S/o Anjineya	Arcevarpatti	7774, Namdhari	50	0	0	10.99°N, 76.77°E
		Nandhakumar S/o Jambu	karagadhahalli	Samrad, Nunhems	90	25	0.00	12.29°N, 78.08°E
		Muniyappan S/o Thadhan	Eranahalli	Greengold, Namdhari	90	35	0.40	12.28°N, 78.06°E
		Sivasakthi S/o Palani	Kammalapatti	Radhika, UPL	90	0	0	12.24°N, 78.09°E
		Sabarinathan S/o Raja	Anjehalli	940, Syngenta	90	60	1.07	12.14°N, 77.97°E
		Rajendiran S/o Anjineya	Pappinenacken Halli	Mono, UPL	60	6	0.47	12.14°N, 78.11°E
		Sivaraj S/o Vetraiyan	Pulikkarai	Selvam, Nunhems	110	0	0.5	12.17°N, 78.11°E
		Mani S/o Raj	Balinjara halli	Samrad, Nunhems	90	0	0.93	12.14°N, 77.95°E
3	Dindigul	Kanthasamy S/o Kanthaiyan	Agraharam	Johny, UPL	75	0	0	12.09°N, 78.44°E
		Balamurugan S/o Sakthivel	Tamaraikulam	Greengold, Namdhari	90	11	0.00	10.37°N, 77.91°E
		Jeyaraj S/o Swaminathan	Oddanchatiram	3312, Syngenta	60	0	1.13	10.48°N, 77.75°E
		Murugasen S/o Subbiah	Punnapatti	Mono, UPL	70	29	0.93	10.24°N, 78.19°E
		Raja S/o Raj	Pallapatti	Radhika, UPL	45	00	0.00	10.37°N, 77.95°E
4	Pollachi	Azhagar S/o Perumal	Natham	102, Syngenta	70	37	2.53	10.22°N, 78.22°E
		Ganeshan S/o Subbiah	Nallakulam	Samrad, Nunhems	90	5	0.87	10.22°N, 78.23°E
		Selvaraj S/o Karupannan	Korimedu	7774, Namdhari	60	0	2.03	10.36°N, 77.98°E
		Veerasamy S/o Jeyabal	Muthugoundanur	Radhika,UPL	60	0	1.60	10.79°N, 76.93°E
		Sampath S/o murugan	Kinathukadavu	Selvam, Nunhems	45	7	0.77	10.82°N, 77.01°E
		Ravi S/o palaniyappan	Vadaputhur	Samrad, Nunhems	75	26	1.87	10.79°N, 76.93°E
		Meena S/o Mayandi	Othakalmandapam	3312,Syngenta	90	9	0	10.87°N, 77.00°E
		Prakash S/o muthuswamy	Kothavadi	Greengold, Namdari	90	0	0	10.80°N, 77.05°E
		Ramesh S/o Anjineya	Arasampalayam	Johny, UPL	60	0	2.03	10.84°N, 77.04°E

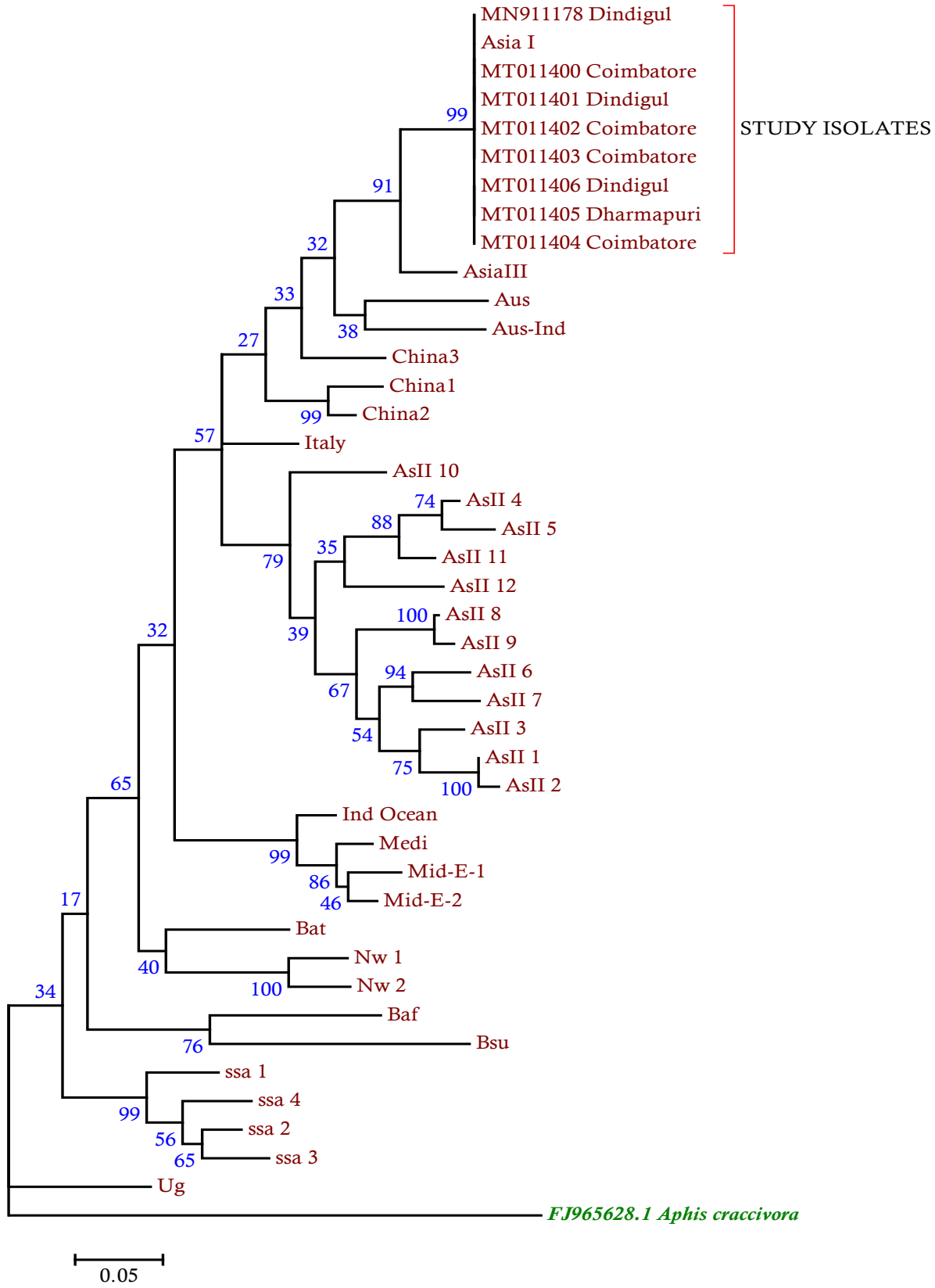

Figure 2. Phylogenetic dendrogram based on mtCOI partial nucleotide sequences of B. tabaci genotypes with numbers at nodes are percentage bootstrap confidence scores (1000 replicates).

In Coimbatore, total mean population of *B. tabaci* (1.82 per plant) and YVMV incidence (13 %) were noticed (Fig 1). The least mean populations were observed on Dharmapuri (0.48 whiteflies per plant) and YVMV incidence (15.75 %).

PCR amplification and sequenced

The genotype of the whitefly population collected from okra leaf sample in eight distinct locations was genetically identified based on *mtCOI* universal primer. Among the eight populations tested for the presence of *mtCOI* gene, all the tested isolates had amplicon of 700 bp. Sequence details of all the isolates showed 99% similarity to *B.tabaci* Asia I and the divergence being less than 3.5%, the threshold value kept for demarcation of the species (de Barro *et al.,* 2011). These PCR amplified sequences were submitted to NCBI and accession number was obtained.(Table 2).

Table 2. Diversity of whitefly genotypes in okra in Tamil Nadu

S.No.	Location (Village/District)	GIS Coordinates	Sample I.D.	Whitefly Biotype	GenBank Accession No.
1	Madampatti, Coimbatore	10.9698° N, 76.8598° E	TNAU01_Whitefly	Asia I	MT011400
2	Natham, Dindigul	10.2261° N, 78.2295° E	ENT23_Whitefly	Asia I	MT011401
3	Madampatti, Coimbatore	10.9698° N, 76.8598° E	Coimbatore41_CPMB	Asia I	MT011402
4	Kinathukadavu, Dindigul	10.8172° N, 77.0186° E	Pollachi58_CPPS	Asia I	MT011403
5	Thondamuthur, Coimbatore	10.9899° N, 76.8409° E	INT_06	Asia I	MT011404
6	Pappininackenhalli, Dharmapuri	12.1519° N, 78.1279° E	Vector35_Whitefly	Asia I	MT011405
7	Natham, Dindigul	10.2261° N, 78.2295° E	vector35_Whitefly	Asia I	MT011406
8	Madampatti, Coimbatore	10.9698° N, 76.8598° E	TNAU02_Whitefly	Asia I	MN911178

Phylogenetic analysis

Based on the phylogenetic tree constructed (Fig. 2) with the accession numbers *viz.,* MT011400 Coimbatore, MT011401 Dindigul, MT011402 Coimbatore, MT011403 Coimbatore, MT011404 Coimbatore, MT011405 Dharmapuri, MT011406 Dindigul, and MN911178 of Dindigul district were clustered with *B.tabaci* Asia I From the above findings, it is concluded that all okra fields show the presence of homogenous population of whitefly genotypes.

CONCLUSION

The survey and analyses performed in the study provided a detailed information on the various species of whitefly population in Coimbatore, Dharmapuri and Dindigul region of Tamil Nadu. The study revealed the specimens collected from okra belong to Asia 1 genetic group and its highly prevalent genetic group in the region. Hashmi *et al.* (2016) reported that Asia II-1 genetic groups were widely distributed across the Indian Agricultural Research Institute, New Delhi. Interestingly, our study provided evidence for the presence of Asia 1 in okra throughout Tamil Nadu. The present survey and the data from GenBank showed that Asia I genetic group was the most prevalent genetic group in these regions of Tamil Nadu.

CONCLUSION

The present study showed that the total mean population of *B. tabaci* (1.82 per plant) and YVMV incidence (13 %) were noticed. The least mean populations were observed on Dharmapuri (0.48 whiteflies per plant) and YVMV incidence (15.75 %). The present study revealed that population of *B. tabaci* was mainly influenced by the YVMV infection in bhendi field. Present survey and the data from GenBank showed that Asia I genetic group was the most prevalent genetic group in the region of Tamil Nadu. In these phylogenetic results, *B. tabaci* Asia I genotype was recorded in all okra fields, indicating the presence of homogenous population of whitefly genotypes in major geographical areas of Tamil Nadu.

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