International Journal of Tropical Insect Science (2023) 43:1123–1133
https://doi.org/10.1007/s42690-023-01004-8

ORIGINAL RESEARCH ARTICLE

Genotyping of *Bemisia tabaci* (Hemiptera: Aleyrodidae) affirmed a new record of Asia II 7, China 3 and dominance of Asia I cryptic species in Bihar, India

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Received: 25 August 2022 / Accepted: 7 April 2023 / Published online: 24 May 2023

Abstract

Whitefly, *Bemisia tabaci* (Gennadius) is a cryptic species complex that infests various plant species and act as a vector for many plant viruses all over the world. To understand the *B. tabaci* cryptic species diversity in Bihar more comprehensively, interhost and interlocation surveys were conducted during the year 2020-2021. The genetic variability among 29 populations (16 interhost and 13 interlocation) was explored using nuclear markers viz. Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR) along with mitochondrial marker, mtCOI. The dissimilarity coefficients of 29 populations clustered in a dendrogram with RAPD and SSR primers showed that interlocation populations were less diverged than the interhost populations. Bayesian phylogenetic analysis of 657 bp mtCOI sequences identified the presence of four cryptic species viz. Asia I, Asia II 1, Asia II 7 and China 3 belonging to two genetic groups (Asia and China) with high variations in interhost unlike in interlocation. Among the four cryptic species, Asia I was the most prevalent in Bihar, establishing 86.20% of all the sequenced samples and Asia II 7 and China 3 were reported for the first time in Bihar region. We believe that the information generated in this study is important from the perspective of identifying cryptic species diversity and to develop long term pest management strategies.

Keywords *B. tabaci* · Interhost · Interlocation · RAPD · SSR · mtCOI · Cryptic species

Introduction

Silverleaf whitefly, *Bemisia tabaci* (Gennadius), is a polyphagous pest that causes severe damage to more than 600 plant species, directly by feeding and excreting honeydew that causes sooty mould, and indirectly by transmitting more than 200 plant viruses (De Lima et al. 2021). In 1889 it was first discovered in cotton fields in Greece (Cock 1993), and on cotton in Pusa (Bihar, India) during 1905 (Misra and Lamba 1929). Due to its remarkable ability to shift, develop, adapt, and monopolise in new environments, it is regarded as one of the top 100 invasive alien species in the world (Ramos et al. 2018). Studies have also revealed the existence of *B. tabaci* cryptic species, which are morphologically ambiguous but does have distinctive biological, physiological, and genetic variations that have caused its prominence to shift its nomenclature from biotypes (Costa et al. 1991), to races (De Barro et al. 2005), to genetic groups (Boykin et al. 2007) and species (Tay et al. 2013).

To date, globally 46 cryptic species of *B. tabaci* with 4% genetic divergence have been identified under 11 genetic groups (Mugerwa et al. 2018; Lestari et al. 2021; Rehman et al. 2021). While 10 cryptic species (Asia I, Asia II 1, Asia II 5, Asia II 7, Asia II 8, Asia II 11, Asia IV, China 3, China 7 and Middle East Asia Minor 1 (MEAM 1)) have

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been reported from India. Understanding the genetic variation and distribution of *B. tabaci* cryptic species has become incredibly important in light of the current climate change, increase in the domestic transportation of agricultural products, and the intensive pest control techniques.

Therefore, in the current study we firstly conducted an interhost survey and learned that Brinjal host had the highest *B. tabaci* infestation, allowing us to conduct interlocation survey in the Brinjal crop of Bihar. Molecular markers viz. RAPD, SSR, mtCOI were used to study the genetic variability and also for characterizing the morphologically indistinguishable *B. tabaci* cryptic species (Mugerwa et al. 2018).

Materials and methods

Whitefly collection

During the survey, adult whiteflies were randomly collected using a handheld aspirator from underside of the leaves from different hosts in the Pusa region of Bihar and also from brinjal host of different locations in Bihar (Kothia, Pusa, Dhrubgama, Mandai Dih, Mirapur, Madhurapur, Jhakra, Alipur Bihta, Faridpur, Charuipar and Dariapur); one location each from Telangana (Tadikonda) and Andhra Pradesh (Bapatla) states as check to explore the diversity (Table 1; Fig. 1). Adults taken from the same host plant at a sampling location were kept in the same tube, whilst those taken from different host plants were kept in separate tubes with 99.9% ethanol. Samples were identified for confirmation of *B. tabaci* using morphological keys (Calvert et al. 2001; Baig et al. 2015).

Genomic DNA extraction

Genomic DNA was extracted based on the method given by Frohlich et al. (1999) with several modifications. Individual adult whitefly from each population (interhost and interlocation) were selected randomly and inserted into 1.5 µL eppendorf tube and a total of 100 µL of 2% cetyltrimethyl ammonium bromide (CTAB), 10 µL of 20% sodium dodecyl sulphate (SDS), 10 µL of 0.1% 2-mercaptaethanol and 10 µL of proteinase K were added and homogenized using the plastic rods. After being homogenized 400 µL of 2% CTAB was added and incubated at 65 °C for 45 min. After the incubation phenol: choloroform: isoamyl alcohol (25:24:1) was added and shaken gently for 10 sec and centrifuged for 10 min at 14000 rpm. A total of 400 µL of supernatant was taken, transferred into a new tube and added equal volume of chilled Isopropanol and put in a −20 °C for 1 hr. After that, it was centrifuged at 12000 rpm for 8 min. The supernatant was then discarded, added 400 µL of 70% chilled ethanol and centrifuged at 12000 rpm for 5 min. The supernatant was then discarded and dried at room temperature, added 50 µL of TE buffer and stored at −4 °C until used as template for PCR amplification.

DNA amplification

Thermocycler was executed with a 20 µl reaction mixture comprising of 2 µl template DNA (100 ng), 1.5 µl of forward and reverse primers, 8 µl of *Taq* mixture and 8.5 µl of nuclease free water. The following conditions were used to run the thermo cycler: initial denaturation at 95°C for 2.30 sec, 35 cycles of denaturation at 94°C for 45 sec, annealing at (Supplementary 1) for 30 sec and extension at 72°C for 5 min followed by final extension of 72°C for 10 min. The PCR results were then electrophoresed using a 2% agarose gel suspension in TAE buffer with 5 µL ethidium bromide at 100 V for 30 min and PCR products were visualized under UV light by Syngene gel documentation system.

Amplicon scoring and data analysis of RAPD and SSR primers

Gel images acquired with the Syngene gel documentation system were employed to score the data matrix (one and zero for the presence and absence of bands, respectively) with AlphaView SA software. The scored marker data matrix was further used to generate a dendrogram (Sneath and Sokal 1973) based on genetic dissimilarity in DARwin 6 software (Perrier et al. 2003). As a result, total amplified bands, number of polymorphic bands, Percentage of Polymorphic Bands (PPB), Polymorphism Information Content (PIC) (Anderson et al. 1993); Resolving Power (RP) (Prevost and Wilkinson 1999); Effective Multiplex Ratio (EMR) (Kumar et al. 2009); Marker Index (MI) (Powell et al. 1996) for each RAPD and SSR marker were computed to determine the informativeness of primers. The genetic variations among interhost and interlocation *B. tabaci* populations analyzed using RAPD and SSR primers (1st tier) were promoted to the 2nd tier (Universal primer: mtCOI analysis).

Sequencing and phylogenetic analysis

The PCR products (mtCOI) were purified using a QIA quick PCR purification kit (Qiagen Inc. Valencia, CA and USA) and then directly sequenced by ABI 3130XL genetic analyzer at Eurofins Genomics Bengaluru, Karnataka. The obtained sequences (657 bp) were aligned in Molecular Evolutionary Genetic Analysis Version X (MEGA X) using ClustalW to look for duplicates, gaps, indels and pseudogenes (Tamura et al. 2011). By performing maximum likelihood fits of 24 different nucleotide substitutions, the best model for phylogenetic tree construction was estimated with help of Bayesian Information Content (BIC) value (Felsenstein 1981). Further, estimates of evolutionary divergence between

Table 1 Details of interhost and interlocation survey conducted during the year 2020-2021

Sl. No	Accession number	Host plant/ Location name	Survey location	District	State	Date of survey	Latitude	Longitude	Altitude/m
1	MZ148550	Congress grass	Pusa	Samastipur	Bihar	05-10-2020	25°59'05.4"N	85°40'50.5"E	53.04 m
2	MZ148551	Indian jujube	Pusa	Samastipur	Bihar	05-10-2020	25°59'05.4"N	85°40'50.5"E	53.04 m
3	MZ148552	French bean	Pusa	Samastipur	Bihar	07-10-2020	25°59'05.4"N	85°40'50.5"E	53.04 m
4	MZ148553	Tomato	Pusa	Samastipur	Bihar	07-10-2020	25°59'05.4"N	85°40'50.5"E	53.04 m
5	MZ148554	Potato	Pusa	Samastipur	Bihar	07-10-2020	25°59'05.4"N	85°40'50.5"E	53.04 m
6	MZ148555	White fig	Pusa	Samastipur	Bihar	12-11-2020	25°59'05.4"N	85°40'50.5"E	53.04 m
7	MZ148556	Chinese hibiscus	Pusa	Samastipur	Bihar	12-11-2020	25°59'05.4"N	85°40'50.5"E	53.04 m
8	MZ148557	Common jasmine	Pusa	Samastipur	Bihar	01-10-2020	25°59'05.4"N	85°40'50.5"E	53.04 m
9	MZ148558	Black nightshade	Pusa	Samastipur	Bihar	01-10-2020	25°59'05.4"N	85°40'50.5"E	53.04 m
10	MZ148559	Barberton daisy	Pusa	Samastipur	Bihar	01-10-2020	25°59'05.4"N	85°40'50.5"E	53.04 m
11	MZ148560	Mexican marigold	Pusa	Samastipur	Bihar	01-10-2020	25°59'05.4"N	85°40'50.5"E	53.04 m
12	MZ148561	Cluster fig	Pusa	Samastipur	Bihar	12-11-2020	25°59'05.4"N	85°40'50.5"E	53.04 m
13	MZ148562	Pointed gourd	Pusa	Samastipur	Bihar	19-02-2021	25°59'05.4"N	85°40'50.5"E	53.04 m
14	MZ148563	Cucumber	Pusa	Samastipur	Bihar	19-02-2021	25°59'05.4"N	85°40'50.5"E	53.04 m
15	MZ148564	Dolichos bean	Pusa	Samastipur	Bihar	19-02-2021	25°59'05.4"N	85°40'50.5"E	53.04 m
16	MZ148565	Okra	Pusa	Samastipur	Bihar	01-10-2020	25°59'05.4"N	85°40'50.5"E	53.04 m
17	MZ148566	Brinjal	Kothia	Samastipur	Bihar	01-10-2020	25°51'17.7"N	85°37'03.5"E	20.42 m
18	MZ148567	Brinjal	Madhurapur	Samastipur	Bihar	05-10-2020	25°58'37.6"N	85°44'09.1"E	26.74 m
19	MZ148568	Brinjal	Dhrubgama	Samastipur	Bihar	05-10-2020	25°58'11.4"N	85°47'41.1"E	44.63 m
20	MZ148569	Brinjal	Pusa	Samastipur	Bihar	01-10-2020	25°59'05.4"N	85°40'50.5"E	53.04 m
21	MZ148570	Brinjal	Mirapur	Muzaffarpur	Bihar	04-10-2020	26°01'42.8"N	85°33'57.6"E	54.16 m
22	MZ148571	Brinjal	Mandai Dih	Vaishali	Bihar	01-10-2020	25°50'16.2"N	85°30'04.6"E	51.53 m
23	MZ148572	Brinjal	Jhakra	East Champaran	Bihar	04-10-2020	26°33'27.9"N	84°57'51.6"E	64.09 m
24	MZ148573	Brinjal	Alipur Bihta	Patna	Bihar	03-01-2021	25°27'19.1"N	85°27'43.4"E	41.11 m
25	MZ148574	Brinjal	Faridpur	Sheikhpura	Bihar	02-10-2020	25°10'58.5"N	85°46'32.1"E	45.11 m
26	MZ148575	Brinjal	Charuipar	Nalanda	Bihar	03-01-2021	25°17'10.1"N	85°27'30.6"E	54.41 m
27	MZ148576	Brinjal	Dariapur	Lakhisarai	Bihar	02-10-2020	25°13'01.3"N	86°04'19.7"E	26.42 m
28	MZ148577	Brinjal	Tadikonda	Mahbubnagar	Telangana	23-11-2020	16°38'59.9"N	78°00'36.7"E	425.7 m
29	MZ148578	Brinjal	Bapatla	Guntur	Andhra Pradesh	23-01-2021	15°56'03.7"N	80°29'42.2"E	121.21 m

sequences and maximum composite likelihood of nucleotide substitution pattern were computed in MEGA X. To ensure proper reading frame, the sequences were translated into their corresponding amino acids using ExPASy translate (Gasteiger et al. 2003), then aligned with ClustalW to observe for conserved, semi-conserved, and fully conserved regions (Sievers et al. 2011). Neutrality tests, such as Fisher's (Fisher 1935) and Tajima's (Tajima 1989) were employed to determine whether the COI fitted to the neutrality requirements. The mtCOI generated sequences were found to be 100% identical to *B. tabaci* and were submitted in National Center for Biotechnology Information (NCBI) GenBank database (Altschul et al. 1997) and accession numbers were retrieved for all the populations (Table 1).

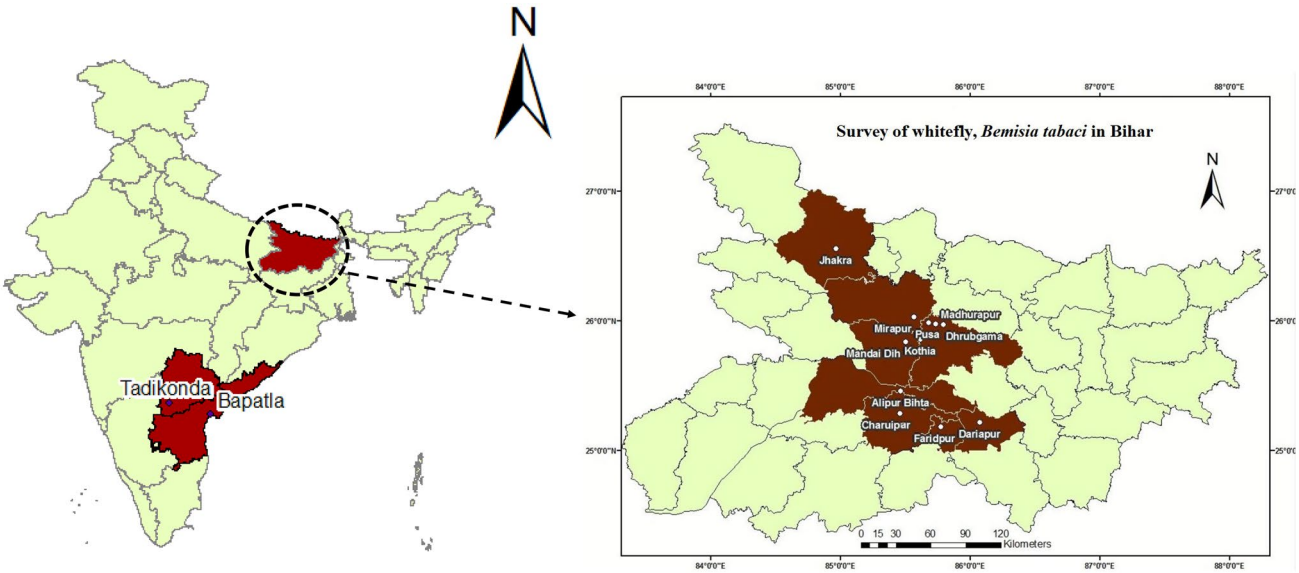

Fig. 1 Survey during the year 2020-2021

Results and discussion

RAPD analysis (1st tier)

RAPD polymorphism

The genetic variation of *B. tabaci* populations was explored with 11 RAPD primers, which were amplified with polymorphism ranging from 90 to 100% and produced 110 bands altogether with mean number of total bands and polymorphic bands per primer was 10.00 and 9.09 respectively. Higher PIC (0.81) in F2 and higher EMR (14.00), MI (10.42) and RP (8.07) in F12 primers revealed there greater informativeness and low EMR (7.00), RP (2.82) in OPA-15; low PIC (0.49), MI (4.45) in OPA-5 revealed there lesser informativeness in examining variation of *B. tabaci* populations (Table 2). Among all the primers, OPA-11 was identified as a potential genetic marker owing to its single monomorphic band with 90% polymorphism, because if there was no monomorphic band, then population would be deliberated as a distinct species (Maurya et al. 2020). Queiroz et al. (2017) observed more than 70% polymorphism in OPA-05 (70.0), OPA-10 (77.9), OPA-11 (73.8), OPA-13 (77.3), OPA-15 (70.8) and these observations are in line with our findings.

Table 2 Details on amplification of RAPD region in genomic DNA of 29 *B. tabaci* populations

Sl. No	Primers	T(°C)	TB	PB	MB	PPB (%)	PIC	EMR	MI	RP
1	OPA-02	37.3	12	12	00	100.00	0.63	12.0	7.61	5.86
2	OPA-04	37.3	09	09	00	100.00	0.67	09.0	6.06	5.79
3	OPA-05	37.3	09	09	00	100.00	0.49	09.0	4.45	4.62
4	OPA-10	37.3	09	09	00	100.00	0.50	09.0	4.52	4.90
5	OPA-11	37.3	10	09	01	90.00	0.61	08.1	4.93	3.69
6	OPA-13	37.3	11	11	00	100.00	0.72	11.0	7.87	5.02
7	OPA-15	37.3	07	07	00	100.00	0.69	07.0	4.89	2.82
8	OPA-20	37.3	09	09	00	100.00	0.61	09.0	5.41	5.59
9	OPR-07	37.3	09	09	00	100.00	0.81	09.0	7.28	4.61
10	F2	37.3	11	11	00	100.00	0.82	11.0	9.02	4.75
11	F12	37.3	14	14	00	100.00	0.74	14.0	10.42	8.07
	Total	-	110	109	-	-	-	-	-	-
	Mean	-	10.0	9.09	-	-	0.663	108.1	6.586	5.06

T (°C) Annealing temperature, *TB* Total Band, *PB* Polymorphic Band, *MB* Monomorphic Band, *PPB* (%) Percentage of Polymorphic Band, *PIC* Polymorphism Information Content, *EMR* Effective Multiplex Ratio, *MI* Marker Index, *RP* Resolving Power

Similarly, Hameed et al. (2012) and Hopkinson et al. (2020) employed RAPD primers to identify variations in *B. tabaci* populations.

UPGMA clustering and dendrogram

The data were clustered using the methodology of the Unweighted pair-group method with arithmetic averages (UPGMA) in a dendrogram, the dissimilarity coefficients of 29 populations. There were ten distinct clusters in the interlocation population, with Bapatla (cluster IV) having the highest dissimilarity coefficient of 46% and Madhurapur and Mirapur (cluster I) having the closest relationships with a dissimilarity coefficient of 30%. Interhost populations of Okra and Dolichos bean (cluster VI) are most closely related with a dissimilarity coefficient of 36% and Common jasmine (cluster X) populations shared higher dissimilarity coefficient of 57%. Cluster I of interlocation population viz. Madhurapur, Mandai Dih, Mirapur, Dhrubgama, Jhakra and Kothia (Northern Bihar) (Fig. 2) and cluster II of Charuipar, Faridpur and Dariapur (Southern Bihar) demonstrates that populations were differentiated based on their geographical locations. Cluster V of interhost populations belong to the Solanaceae family shared a lesser dissimilarity coefficient (37%). Results clearly demonstrated that interlocation populations were less diverged than the interhost populations, due to the fact that they were primarily collected from the same host viz. brinjal. Similar pattern studies were conducted with RAPD primers in *B. tabaci* (De Barro and Driver 1997); *Myzus persicae* (Zitoudi et al. 2001); *Helicoverpa armigera* (Lopes et al. 2017); *Leucinodes orbonalis* (Murali et al. 2021).

SSR analysis (1st tier)

SSR polymorphism

The genetic diversity of *B. tabaci* populations was investigated using nine SSR primers, which amplified with 100% polymorphic bands and generated a total of 60 bands, with 6.66 mean number of total bands and polymorphic bands. Higher PIC (0.878), EMR (9.0), MI (7.902) and RP (5.79) in Btls1-2 revealed there greater informativeness and low PIC (0.664) in Bta1; EMR (4.00) and MI (3.196) in Btls 1-6 and RP (1.238) in Bta 11 revealed less informativeness in examining the variability of *B. tabaci* populations (Table 3). Similar to our studies, De Barro et al. (2003), Simón et al. (2007), Gauthier et al. (2008) and Ben Abdelkrim et al. (2017) used these primers for examining genetic variability among *B. tabaci* populations. Contrarily, Valle et al. (2012) observed lowest polymorphism percentage in Bta11 primer, which showed 100% in our study.

UPGMA clustering and dendrogram

The UPGMA technique was used to cluster the data into a dendrogram using the dissimilarity coefficients of 29 populations. There were ten distinct clusters in the interlocation population, with Alipur Bihta, Dhrubgama, and Mirapur (cluster V) having the highest dissimilarity coefficient of 42% (cluster VIII) and being the most closely connected with a dissimilarity value of 30%. However, interhost populations of Cucumber and Potato (cluster I) are most closely related, sharing a dissimilarity coefficient of

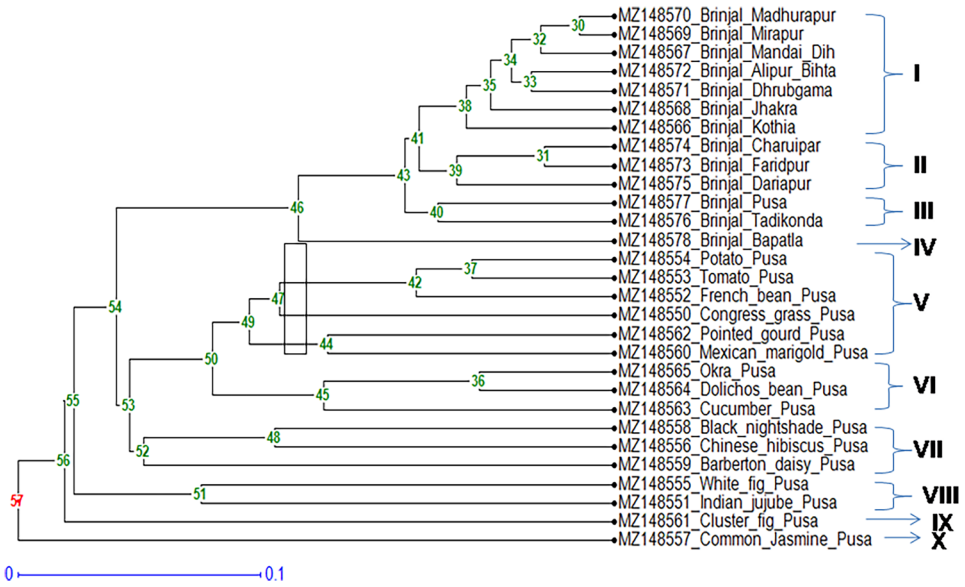

Fig. 2 Dendrogram deduced from matrix of pair wise distances in RAPD analysis of *B. tabaci* interhost and interlocation using UPGMA

Table 3 Details on amplification of SSR region in genomic DNA of 29 *B. tabaci* populations

Sl. No	Primers	T(°C)	TB	PB	MB	PPB (%)	PIC	EMR	MI	RP
1	Btls1-2	50.0	09	09	00	100.00	0.88	09	7.90	5.79
2	Btls1-6	50.0	04	04	00	100.00	0.79	04	3.19	1.38
3	Bta1	51.0	09	09	00	100.00	0.66	09	5.98	4.38
4	Bta4	51.0	06	06	00	100.00	0.69	06	4.19	3.45
5	Bta11	50.0	05	05	00	100.00	0.82	05	4.08	1.24
6	Bta12	51.0	06	06	00	100.00	0.68	06	4.11	3.73
7	BEM 12	53.0	06	06	00	100.00	0.84	06	5.03	2.27
8	BEM 23	53.0	07	07	00	100.00	0.84	07	5.91	1.58
9	BEM 37	51.0	08	08	00	100.00	0.86	08	6.86	4.41
	Total	-	60	60	-	-	-	-	-	-
	Mean	-	6.66	6.66	-	-	0.79	6.66	5.25	3.13

T (°C) Annealing temperature, *TB* Total Band, *PB* Polymorphic Band, *MB* Monomorphic Band, *PPB* (%) Percentage of Polymorphic Band, *PIC* Polymorphism Information Content, *EMR* Effective Multiplex Ratio, *MI* Marker Index, *RP* Resolving Power

38%, while, Common jasmine was the most divergent with higher dissimilarity coefficient of 56% (Fig. 3). According to Fakrudin et al. (2004), this augmented genetic variability might aid species in evolving and adapting to new environment more quickly. The lower dissimilarity coefficient observed between *B. tabaci* populations from interlocation can be enlightened by the certainty that they were all collected from the same host (Brinjal), whereas the higher dissimilarity coefficient observed among *B. tabaci* populations from interhost could be due to their collection from different hosts. Similar pattern of differentiation studies were conducted by Valle et al. (2012) and Reddy et al. (2022) using SSR primers in *Bemisia tabaci* and *Helicoverpa armigera* populations, respectively.

mtCOI analysis (2nd tier)

To construct a *B. tabaci* cryptic species phylogenetic tree, we first collected 29 mtCOI sequences from interhost and interlocation of Bihar along with Andhra Pradesh (Bapatla) and Telangana (Tadikonda) produced an amplicon of 657 bp mtCOI region (Supplementary 1). The phylogenetic tree was built based on Hasegawa-Kishino-Yano with Gamma distribution model (HKY+G) for 29 sequences along with 44 reference sequences and *Bemisia atriplex*, *Bemisia afer* and *Trialeurodes vaporarium* as out groups (Fig. 4) (Supplementary 2).

It was noted that four cryptic species viz. Asia I, Asia II 1, Asia II 7 and China 3 were found to cluster with 29

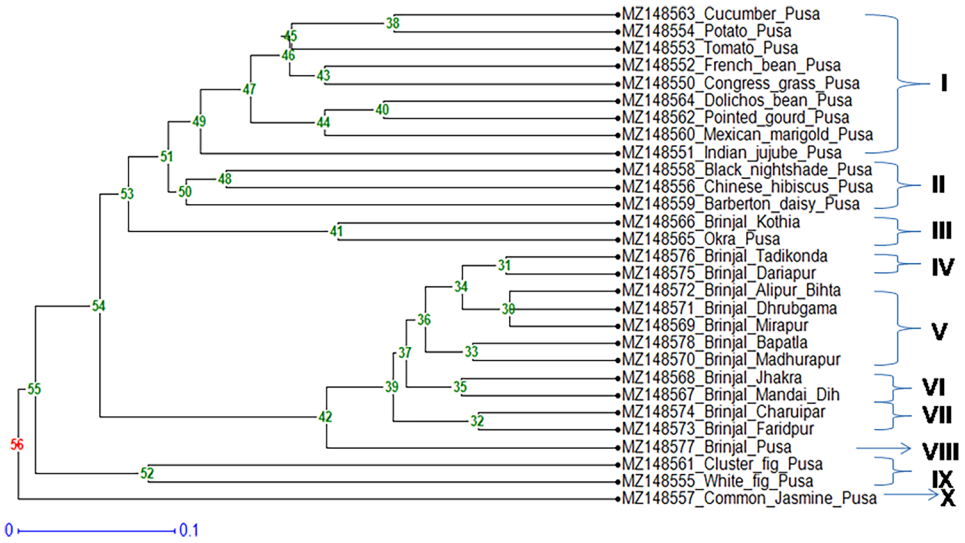

Fig. 3 Dendrogram deduced from matrix of pair wise distances in SSR analysis of *B. tabaci* interhost and interlocation using UPGMA

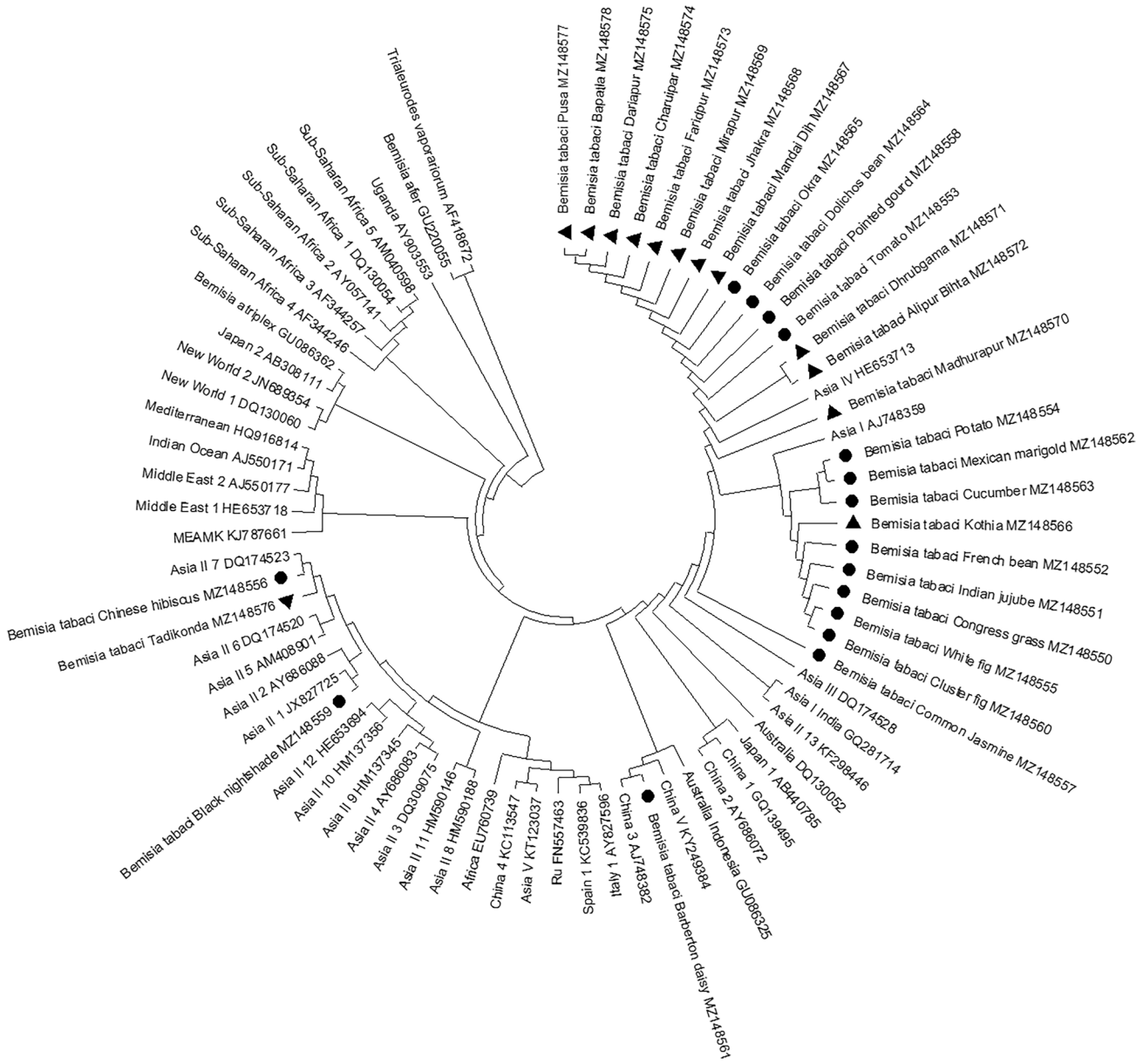

Fig. 4 Phylogenetic tree inferred from 657 bp sequences of 29 mtCOI genes, 44 cryptic species of *B. tabaci* and three out groups

B. tabaci populations (Fig. 5). The sequences of interhost (Okra, Dolichos bean, Pointed gourd, Tomato, Potato, Mexican marigold, Cucumber, French bean, Indian jujube, Congress grass, White fig, Cluster fig and Common jasmine); interlocation (Pusa, Bapatla, Dariapur, Charuipar, Faridpur, Mirapur, Jhakra, Mandai Dih, Dhrubgama, Alipur Bihta, Madhurapur, Kothia) clustered with Asia I was found to be the major cryptic species accounting for 25 of the 29 (86.20%) *B. tabaci* populations. And therefore, Asia I had a greater potential to inflate and adapt in Bihar among four reported cryptic species. Similarly, Roopa et al. (2015) sequenced 71 *B. tabaci* samples and found Asia I cryptic species to be the most predominant accounting for 44 out of the 71 samples (61.97%).

Asia II is a genetically diverse group consisting of 13 sub cryptic species, Asia II (1–13) (Kanakala and Ghanim 2019) among them, only Asia II 1 and Asia II 7 were detected from the collected *B. tabaci* samples. Interestingly, Black nightshade and Chinese hibiscus collected from single location (Pusa region) clustered with Asia II 1 and Asia II 7, respectively shows that cryptic species add a impact on host plant selection. Roopa et al. (2015) discovered Asia II 7 cryptic species on Chinese hibiscus, which supports with our findings. Chowda-Reddy et al. (2012) stated that Asia II 7 was

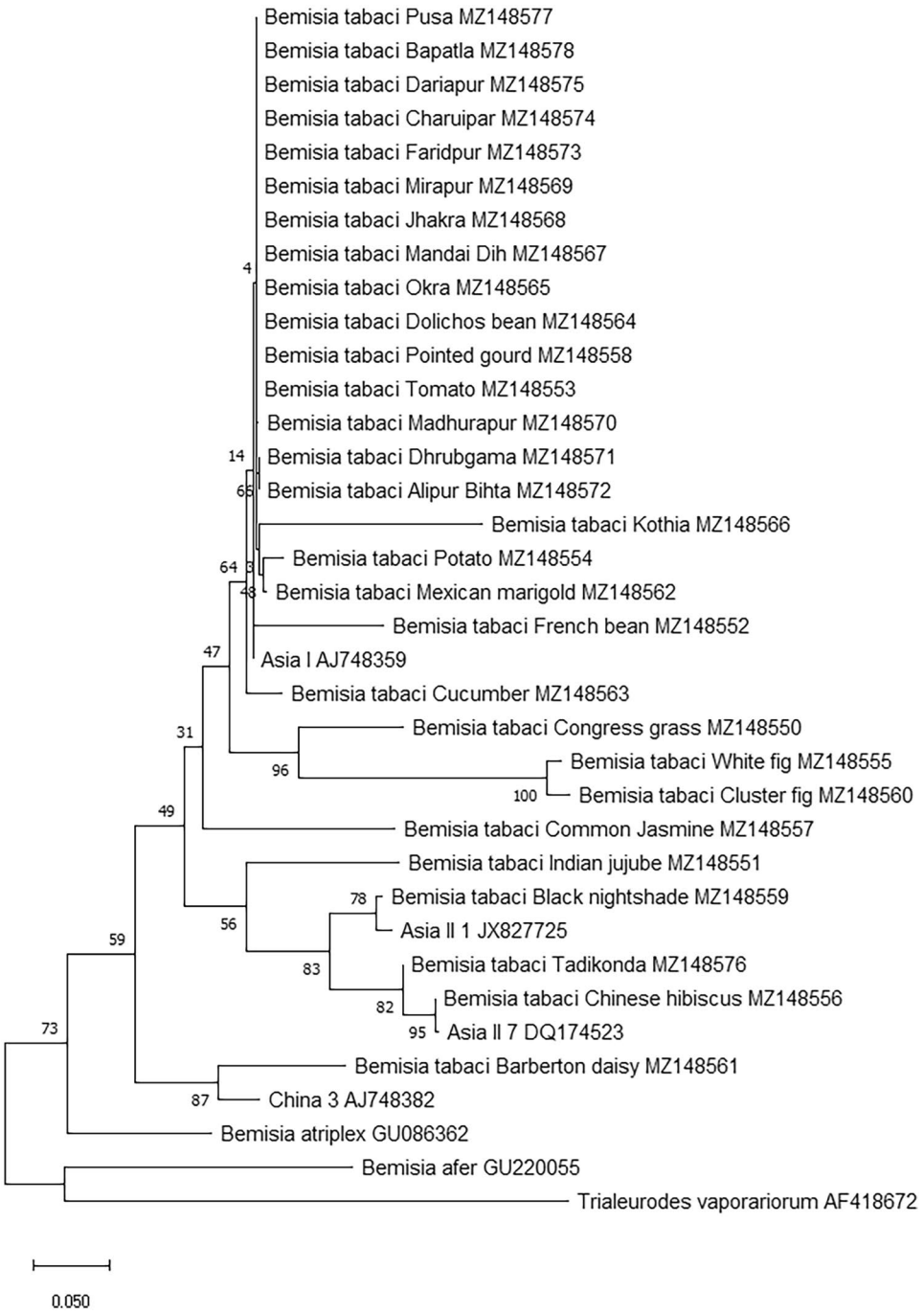

Fig. 5 Phylogenetic tree inferred from 657 bp sequences of 29 mtCOI genes, four cryptic species of *B. tabaci* and three out groups

primarily found in Southern and Western India, which is in contrast to our results as Asia II 7 cryptic species was found in Bihar (Eastern India). Moreover, Barberton daisy grouped with China 3 (Fig. 4) has been discovered for the first time in Bihar, which borders West Bengal and is where Ellango et al. (2015) first discovered the China 3 cryptic species.

In contrast to the prior data given by Misra and Lamba (1929), Chowda-Reddy et al. (2012), Roopa et al. (2015) and Rangaswamy et al. (2019), it was evident that among the four cryptic species (Asia I, Asia II 1, Asia II 7, and China 3); Asia II 7 and China 3 were reported for the first time in the Bihar region. Given the wide-spread occurrence of numerous cryptic species, the abundance of suitable hosts, climate change, the overall domestic transportation of agricultural products, and intensive pest control strategies, *B. tabaci* has a high likelihood of acquiring an adaptive advantage in various parts of the nation.

Multiple sequence alignment

Multiple alignment of 29 *B. tabaci* nucleotide sequences revealed 305 completely conserved residues (Supplementary 5) and 105 Single Nucleotide Polymorphisms (SNPs). Furthermore, in multiple alignment of amino acid sequences 75 fully conserved residues, 46 conserved residues and 23 semi conserved residues were identified (Supplementary 6). This greater level of nucleotide similarity indicates that they evolved from a common origin. Similarly, Wosula et al. (2017); Kunz et al. (2019) found 7453 SNPs and 125 conserved amino acid residues, respectively with amplification of mtCOI region of *B. tabaci*.

Pair wise genetic distance

The pair wise genetic distance of *B. tabaci* populations ranged from 0.00 to 0.47 (Supplementary 4) with an over mean distance of 0.08. Similarly, Dinsdale et al. (2010) reported zero to 34% genetic distance among 198 *B. tabaci* populations.

Patterns of nucleotide substitution in mtCOI

As nucleotide composition is a crucial aspect of nucleic acids, the study revealed that thiamine (T) (43.37%) and guanine (G) (19.00%) had the highest and lowest numbers of nucleotide bases, respectively. Similarly, Roopa et al. (2015) observed highest nucleotide base in thiamine (43.10%) and lowest in guanine (13.22%) with *B. tabaci* mtCOI sequences. Furthermore, the base composition of mtCOI gene fragment was biased towards Adenine (A) and Thymine (T) with an overall 67.54% which was a universal feature of nucleotide diversity (Lynch 2008).

Neutrality tests

The negative Tajima's $D = -0.726310$ (Table 4) and Fisher's exact test with P value <0.05 (Common jasmine, Congress grass, Chinese hibiscus, Black nightshade, Cucumber, Kothia, Madhurapur, Dhrubgama, Alipur Bihta, White fig, Chinese hibiscus and Tadikonda) (Supplementary 7) indicated excess of low frequency polymorphisms. Thus, both tests supported the neutral theory of evolution and these findings were supported by Tocko-Marabena et al. (2017) who concluded that *B. tabaci* was found to be significant with Tajima's D (-2.45317).

Table 4 Results from Tajima's Neutrality Test

m	*S*	p_s	Θ	Π	*D*
36	402	0.611872	0.147554	0.119309	-0.726310

m number of sequencesm, *S* Number of segregating sites, $p_s = S/n$, $\Theta = p_s/a_1$, π nucleotide diversity, *D* is Tajima test statistic

Conclusion

In the present study, we were able to confirm the existence of four *B. tabaci* cryptic species (Asia I, Asia II 1, Asia II 7, and China 3) in Bihar. In particular, Asia II 7 and China 3 were discovered for the first time in the Bihar region, while Asia I cryptic species dominated all interhost and interlocation populations. Overall, this study contributes to the characterization of various *B. tabaci* cryptic species for assessing ongoing changes in genetic diversity, evolutionary history, and potential spread that enable effective pest management while avoiding overuse of insecticides and lowering environmental pressure.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42690-023-01004-8.

Acknowledgement The authors are grateful to Department of Entomology, Department of Genetics and Plant Breeding, and Department of Biotechnology, Dr. Rajendra Prasad Central Agricultural University for their unwavering support throughout the research. This research is part of Gummudala Yashaswini M.Sc dissertation.

Data availability All data relevant to the study are included in the article.

Declarations

Conflict of interest The authors have no competing interests to declare.

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