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Unravelling genetic diversity of whitefly species on different host plants from New Delhi

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Abstract

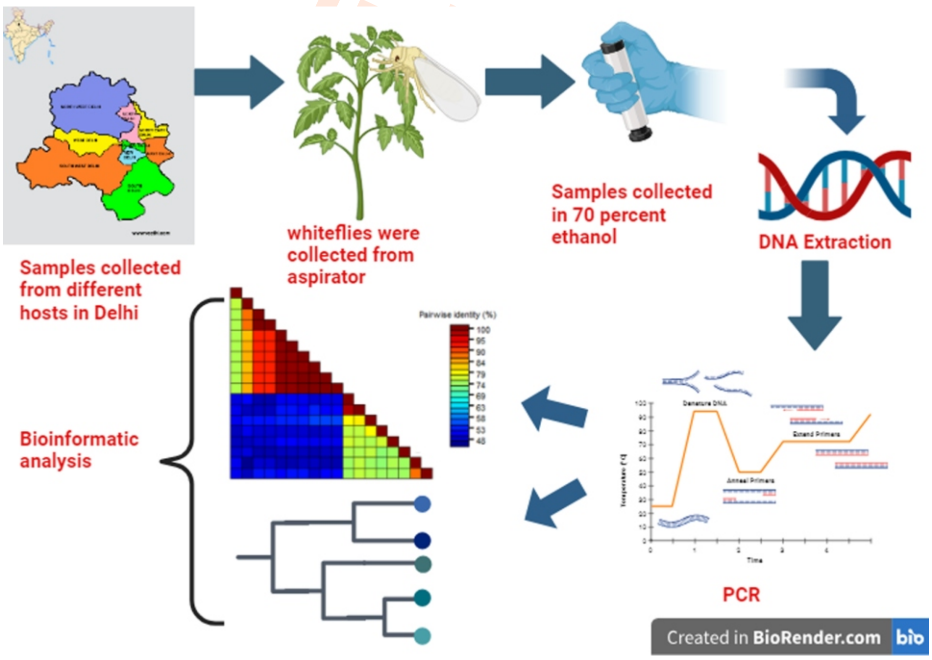
Aim: This study investigates the genetic diversity and distribution of different species of whiteflies on 20 host plants vegetables (cucumber, summer squash, pumpkin, tomato, brinjal, okra), field crops (sunflower, lettuce, field bean, lobia), commercial crops (cotton, tobacco), ornamentals (chrysanthemum, bellflower, marigold), fruit crops (lemon), trees (mulberry, wild tamarind, ficus), and others (wild brinjal) in New Delhi.

Methodology: Genetic diversity was assessed through mitochondrial cytochrome oxidase-I gene-based molecular markers.

Results: Phylogenetic analysis revealed that whitefly *Bemisia tabaci* was the most prevalent pest species across the crops reported in this study. The other whitefly species, viz., *Dialeurodes* sp., *Singhiella simplex*, *Aleuroclava* sp., *Tetraleurodes acaciae*, *Trialeurodes vapororium,* and *Aleurodicus floccissimus* were reported as pests of different horticultural crops.

Interpretation: Genetic diversity of *B. tabaci* revealed that the genetic groups Asia II-1 and Asia II-7 were most prevalent, while Asia I was the minor one. Understanding the species composition and genetic diversity of whiteflies will help in devising appropriate control strategies.

Key words: Hosts, Molecular markers, mtCOI, New Delhi, Phylogenetic analysis, Whitefly diversity



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Introduction

Whiteflies are small white-winged appearance, that cause damage to the crops both as plant pests and disease vectors. As pests, they feed on plant sap, leading to stunted growth, yellowing of leaves, and plant deterioration. Their role as vector contributes to the transmission of plant diseases, posing a dual threat to horticultural crops. The direct damage weakens the plant health, reducing yields, while the transmission of viruses further compromises production and quality attributes. This includes deformed or discoloured fruits, decreased marketability, and alterations in taste and nutritional value. Effective pest management is essential to mitigate these detrimental impacts and sustain horticultural crop production (Sundararaj *et al.,* 2021; Kumar *et al*., 2018 and Chandrashekar *et al*., 2020). Whiteflies, often introduced accidentally with their host plants, are successful invaders due to their small size, cryptic nature, and the transportation of their immature stages with host plants. They exhibit rapid multiplication, high phenotypic plasticity, and the potential to outcompete native species, causing damage to economically vital crops (Sundararaj *et al.,* 2021 and Simala *et al.,* 2015).

In India, 469 whitefly species from 71 genera, including eight invasive species, are known to infest agricultural, horticultural, and forestry crops (Sundararaj *et al.,* 2021 and Mohan *et al.*, 2019). India witnessed its first invasive whitefly, the spiralling whitefly (*Aleurodicus dispersus*), in the Western Ghats in 1995. Subsequently, between 2015 and 2019, seven more exotic whiteflies were identified, posing threats to various crops. These include the solanum whitefly, rugose spiralling whitefly, legume-feeding whitefly, Bondar's nesting whitefly, nesting whitefly, palm infesting whitefly, and woolly whitefly. These invasive whiteflies belong to the subfamilies Aleurodicinae and Aleyrodinae. Whiteflies, particularly *Bemisia tabaci,* rank as the 14th worst invasive species globally, posing a significant threat to agriculture. This species is part of a vast family with 1556 species across 161 genera, infests over 1000 plant species critical for food, fibres, and aesthetics (Sani *et al.,* 2020). *B. tabaci* is notorious for transmitting more than 300 plant-pathogenic viruses, making it a major agricultural menace. Rehman *et al.* (2021) identified nine cryptic species of *B. tabaci* in India, with distinct regional prevalence.

The southern region harbours Asia 1, Asia II 5, and Asia II 8, while the northern and central parts exhibit Asia II 1 and Asia I. Western India records the highest numbers of *B. tabaci* Asia I and Asia II 7 instances whereas, in the Eastern India Asia I, Asia II 1, and Asia II 5 are prevalent. Whitefly infestation results in visible symptoms like chlorotic leaf spots and the growth of sooty fungi, further impairing plant health. Genetic research by Rehman *et al.* (2021) revealed genetic divergence ranging from 4.1% to 23.4% among cryptic species and 11.7% to 21.2% within genetic groups of *B. tabaci.* A paradigm shift from biotype to cryptic species identification was prompted by De Barro *et al.* (2011), who revised the divergence threshold to 4%. The Asia II 1 genotype exhibits wide prevalence and high haplotype diversity across diverse climatic regions in the Indian subcontinent, while the Asia 1 genotype showcases superior nucleotide diversity. Genetic clusters in Delhi include Asia II 1 and Asia II 7 (Prabhulinga *et al.,* 2021). Understanding species diversity and genetic variation is crucial for effective management strategies against the evolving *B. tabaci* cryptic species complex and other whitefly genera. The Old-World genus *Aleuroclava* is primarily found in the Oriental and Australasian regions, with a total of 62 species, and is also found in India (Pushpa and Sundararaj, 2010). With about 100 species, it is distributed in the Palaearctic Regions. *Trialeurodes vaporariorum*, the glasshouse or greenhouse whitefly, is a widespread insect in temperate regions, measuring 1-2 mm, thriving as a primary pest in protected horticultural environments. All stages, except eggs and pupae, contribute to plant damage by feeding on phloem sap, with honeydew excretion exacerbating harm. Adults, pivotal in transmitting plant viruses, notably impact crops such as cucurbits, potatoes and tomatoes (Manzano, 2009).

Aleurodicus is a genus of whiteflies recognised for large size compared to other whitefly genera, with wingspans ranging from a few millimetres to over a centimetre. These insects, commonly known as giant whiteflies with size 1/12-1/8 inch, are global in distribution and infest a broad range of host plants, including agricultural crops and ornamental species. *Aleurodicus* species are known for feeding on plant sap, causing damage such as reduced plant vigour and yellowing of leaves (Kalyanasundaram and Mani, 2022). One notable species, *Aleurodicus floccissimus,* is characterised by distinctive fluffy or cottony wax secretions covering its body (Rao *et al.,* 2018). The globally distributed genus *Tetraleurodes* Cockerell (Hemiptera: Aleyrodidae), comprising around 70 species, has been identified in various geographical regions. Tetraleurodes species feed on both monocot and dicot plants, and certain species, such as those in the *Tetraleurodes acaciae* group, are associated with legumes (Fabaceae). The puparium of legume-feeding species displays a series of glandular tubercles with a central pore along the submargin (Dubey and Ramamurthy, 2015). *Singhiella simplex* commonly known as fig whitefly is native to India, Myanmar and China. It attacks *Ficus* spp., extending its presence to Asia, the Americas and Europe (Laudani *et al.,* 2020).

While the citrus whitefly (*Dialeurodes citri*) was once a prominent pest in Florida, its significance has now diminished, though it remains a notable concern. Believed to have been introduced from India between 1858 and 1885, it is prevalent in Florida, parts of the Gulf States, California, and U.S. greenhouses. It has a global distribution spanning Africa, Asia, Central America, the Caribbean, the Commonwealth of Independent States, Europe, North America and South America. The small mealy-white adult boasts four wings covered in a powdery white wax, leading to its moniker "cloudy-winged whitefly" (Barbagallo *et al.,* 2022). In view of the above, the objective of this study lies in understanding the diversity of *B. tabaci* and other whitefly genera on diverse crops within the Delhi region, leveraging mitochondrial subunit analyses to pave the way for comprehensive and strategic pest management approaches.

Materials and Methods

The study, conducted from 2021 to 2023, involved the meticulous collection of whiteflies throughout the year, covering different seasons. The collection process included random sampling, wherein a 'Z' pattern was employed to cover blocks of crops spanning at least two hectares. Insects were gathered using an aspirator in the early morning, along with leaves hosting nymphs and pupae infestations (Naveen *et al*., 2017). Transport to the laboratory was facilitated in ventilated cages, with leaflets placed in damp sponges. Infested leaflets were retained within the cages to allow for the emergence of new adult insects. A total of 57 samples were obtained, with three samples collected from each of the 20 distinct host plants from top five leaves. To ensure a diverse representation, whiteflies were collected from a variety of host plants in Najafgarh and IARI arm, New Delhi (collection details vide Table 1). These plants included vegetable crops (cucumber, summer squash, pumpkin, tomato, brinjal, and okra); field crops (sunflower, lettuce, field bean, and lobia); commercial crops (cotton and tobacco); ornamentals (chrysanthemum,

Table 1: Survey locations and host plants of whitefly populations

Host (Scientific name)	Location	GPS coordinates
Field bean - *Vicia faba*	IARI Farm, New Delhi	28.641955 N, 77.162572 E
Chrysanthemum - *Chrysanthemum* spp.	IARI Farm, New Delhi	28.62579 N, 77.16064 E
Cotton - *Gossypium hirsutum*	IARI Farm, New Delhi	28.640982 N, 77.162448 E
Tomato - *Solanum lycopersicum*	IARI Farm, New Delhi	28.641955 N, 77.162572 E
Brinjal (Eggplant) - *Solanum melongena*	IARI Farm, New Delhi	28.64101 N, 77.16984 E
Marigold - *Tagetes* spp.	IARI Farm, New Delhi	28.641809 N, 77.160920 E
Okra - *Abelmoschus esculentus*	IARI Farm, New Delhi	28.641219 N, 77.164407 E
Cucumber - *Cucumis sativus*	Farmers Field, Najafgarh, New Delhi	28.60616 N, 76.98244 E
Lobia (Black-eyed pea) - *Vigna unguiculata*	IARI Farm, New Delhi	28.641219 N, 77.164407 E
Mulberry - *Morus* spp.	IARI Farm, New Delhi	28.641219 N, 77.164407 E
Bellflower - *Campanula* spp.	IARI Farm, New Delhi	28.62579 N, 77.16064 E
Tobacco - *Nicotiana tabacum*	IARI Farm, New Delhi	28.60616 N, 76.98244 E
Wild brinjal (Wild Eggplant) - *Solanum torvum*	IARI Farm, New Delhi	28.641809 N, 77.160920 E
Summer squash - *Cucurbita pepo*	Farmers Field, Najafgarh, New Delhi	28.60616 N, 76.98244 E
Pumpkin - *Cucurbita moschata*	IARI Farm, New Delhi	28.640982 N, 77.162448 E
Wild tamarind - *Lysiloma latisiliquum*	IARI Farm, New Delhi	28.641809 N, 77.160920 E
Lemon - *Citrus limon*	Farmers Field, Najafgarh, New Delhi	28.60616 N, 76.98244 E
Ficus – *Ficus religiosa*	IARI farm, New Delhi	28.640982 N, 77.162448 E
Sunflower - *Helianthus annuus*	IARI farm, New Delhi	28.641955 N, 77.162572 E
Wild tamarind - *Lysiloma latisiliquum*	IARI farm, New Delhi	28.64101 N, 77.16984 E

Table 2: List of different whitefly species along with their hosts and accession numbers obtained in this study

Whitefly species	Host plants	Accession numbers
Bemisia tabaci Asia 1	Field bean, chrysanthemum	OQ928085
Bemisia tabaci Asia II-1	Cotton, tomato, brinjal	OQ706118
	Marigold, okra, cucumber	MH909094
	Lobia	MH909092
	Mulberry, bellflower	OQ402723
	Tobacoo, wild brinjal	OQ402685
	Summer squash, pumpkin	OQ402683
Bemisia tabaci Asia II-7	Lettuce	MN830435
	Wild tamrind	MN830439
Aleuroclava sp.	Lemon	OQ929662
	Mulberry	OQ706115
Singhiella simplex	Ficus	OQ927591
Aleurodicus floccissimus	Sunflower	OQ927573
Trialeurodes vaporariorum	Cucumber	OQ927979
Tetraleurodes acaciae	Mulberry	OQ927603
Dialeurodes sp.	Ficus	OQ706119

bellflower, and marigold); a fruit crop (lemon); trees (mulberry, wild tamrind (*Leucaena leucocephala*), and ficus); and others like wild brinjal. Subsequently, the collected whiteflies were transferred to 1.5-ml Eppendorf tubes containing 70% ethanol solution. These samples were then preserved at -20°C for subsequent analyses.

For DNA extraction, minimum of five adult individuals was used for each specimen The individual whiteflies were homogenised in 50µl solution of 10% Chelex® 100 Resin (Bio-Rad) using a hand-operated motorized homogenizer under sterile conditions. The homogenized extract was incubated at 56°C for 20 min, followed by a 5 min incubation at 95°C. The resultant mixture was then subjected to centrifugation at a relative centrifugal force of 13,500 for 5 min, and the supernatant was used as a template for polymerase chain reaction analysis. The quality of DNA was ascertained on a 0.8% Agarose gel containing ethidium bromide, and quantification of DNA concentration was done on the Nanodrop (Nabi™). Identification of whitefly species was based on *mtCO1* sequence-based analysis as described by Simon *et al.* (1994). The universal primers C1-J-2195 (5'-TTGATTTTGGTCATCCAGAAGT-3') and TL2-N-3014 (5'CCAATGCACTAATCTGCCATATTA-3') were used to amplify the *mtCOI* gene fragment of approximately 820 bp size.

The PCR reaction mixture consisted of 25 µl, including ready-to-use PCR master mix (Promega M750A), nuclease-free water, forward and reverse primers, and DNA template. The resulting 3-microliter PCR product underwent electrophoresis for 45 min on a 1.2% agarose gel in 1X Tris-acetate-EDTA buffer at 100 volts using equipment from Jordan Scientific. Amplified PCR products were purified and sequenced at Barcode Bioscience in Bengaluru, India. The acquired whitefly sequences were aligned using the ClustalW tool in BioEdit v7.2.5. Subsequently, Mega 11 software was employed for phylogenetic analysis, following the Kimura (1980) and Kumar *et al.* (2018) models. Phylogenetic tree was constructed by the maximum likelihood approach, implementing the Kimura 2-parameter model. To validate the phylogenies, a bootstrap replication of 1000 was performed (Felsenstein, 1985). Taxonomic classification of whitefly sequences was ascertained based on the criteria established by Dinsdale *et al.* (2010), focusing on clades exhibiting a pair-wise sequence divergence exceeding 3.5%. These sequences were then submitted to the National Centre for Biotechnology Information's (NCBI) GenBank to obtain accession numbers. For a comprehensive understanding of genetic parameters, the software Dna SP v5.10 was employed to analyse *B. tabaci* COI sequences. This analysis encompassed sequence polymorphism, singleton variable sites, average nucleotide differences, G+C content, number of haplotypes, haplotype diversity, and nucleotide diversity. The analytical framework followed the methodology outlined by Librado and Rozas (2009). To delineate the haplotype network of *B. tabaci* sequences, the software popART was utilised. This involved creating a minimum-spanning network relationship among the cryptic species, providing valuable insights into the genetic connections within the population.

Table 3: Genetic diversity analysis in mitotypes of *B. tabaci* India

Mitotypes	No. of Sequences	No. of haplotypes	Number of variable sites (S)	Haplotype diversity	Nucleotide diversity (Pi)	Average number of nucleotide differences (k)	G+C content	Total number of mutations (Eta:)	Fu's Fs statistic:	Strobeck's S statistic	Tajima's D	Fu and Li's D* test statistic	Fu and Li's F* test statistic
Asia II 7	2	2	3	1.00	0.00375	3.000000	0.333	3	1.099	1.00	NA	NA	NA
Asia II 1	6	6	9	0.933	0.00458	3.66667	0.335	9	-0.905	0.943	-0.41545	-0.43366 (NS)	-0.46330 (NS)
Asia I	11	11	199	1.00	0.41212	92.72727	0.333	322	0.259	1.00	-0.75795	-0.19567	-0.38706

Results and Discussion

The distribution of whitefly species is widespread across various crops, causing significant damage. Utilising the maximum likelihood methodology, the phylogenetic examination of nine distinct whitefly specimens obtained from various crops revealed a combined total of three distinct mitotypes of *B. tabaci,* specifically Asia-1 on field bean and chrysanthemum; Asia II-7 on lettuce and wild tamarind; and AsiaII-1 on many hosts (Fig. 1, Table 2). Six other whiteflies belonging to different genera were also recorded including *Dialeurodes* sp. and *S. simplex* on ficus, *Aleuroclava* sp. on lemon and mulberry, *T. acaciae* on mulberry, *T. vapourorium* on cucurbits, and *A. floccissimus* on sunflower (Table 2). From phylogeny, it was apparent that the cryptic sequences of *B. tabaci* were grouped together separately from other *Bemisia* species. Two *Aleuroclava* sequences were grouped together with a bootstrap value of 100. The remaining whitefly genus were grouped separately (Fig. 2). Diverse whitefly species impact crops differently, posing threats as vectors of virus diseases. The necessity for frequent insecticide sprays to control these pests not only affects crop quality but also raises concerns about the nutritional status of vegetables and fruits. Studying haplotype diversity in *B. tabaci* genetic groups informs their evolutionary patterns and population dynamics, providing essential insights for effective pest management strategies. Understanding genetic variation aids in identifying adaptive traits and potential vulnerabilities within whitefly populations. A similarity analysis of mitotypes Asia II-1 and Asia II-7 of *B. tabaci,* along with mitotypes Asia-1 and other species of whitefly, was conducted using the Sequence Demarcation Tool Version 1.2 (SDTv1.2), resulting in a colour-coded pair-wise identity matrix. The matrix is depicted in Fig. 3. The results indicated that Asia II-1 sequences showed greater than 95% similarity among them, with more than 70% similarity to other Asiatic groups. This is even more similar with respect to Asia II-7 genetic groups. However, this was not the case with Asia 1, which showed greater than 87% similarity with Asia II-7 sequences and less than 85% similarity with Asia II-1 sequences. This shows that Asia II-7 was more closely related to Asia I than Asia II-1.

Table 2 shows the total number of mutations, G+C content, haplotype diversity, total number of variable sites, nucleotide diversity, average number of nucleotide differences, and number of haplotypes of *B. tabaci* cryptic species identified in New Delhi so far. The nucleotide diversity was found to be higher in Asia I (0.41212) as compared to Asia II-1 (0.00458). However, the haplotype diversity was greater in Asia II-7 and Asia-I (1.00) than in Asia II-1 (0.933). A total of 19 sequences were analysed, from which three haplotypes were identified. In this study, one haplotype was observed for Asia-1, while Asia II-7 exhibited two haplotypes, and the maximum number of haplotypes was observed in Asia II-1, with a total of six haplotypes (Table 3). The remaining

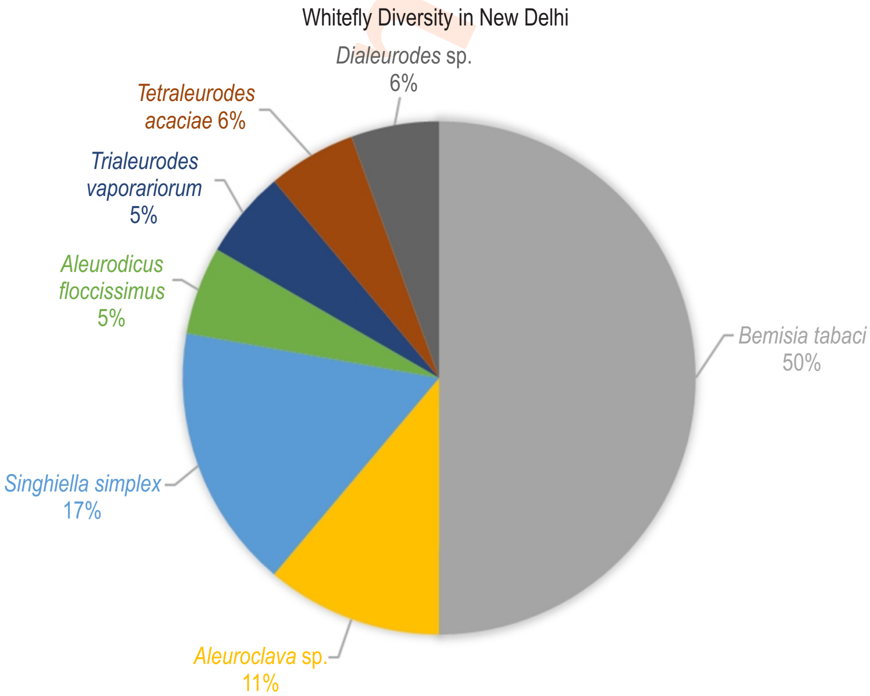

Fig. 1: Pie chart of different whitefly species reported in this study. This pie chart visually represents the distribution of various whitefly species documented in the current study. Each segment of the pie is labelled with the respective whitefly species, and the proportion percentage is indicated to provide a clear representation of their relative abundance in the sampled population.

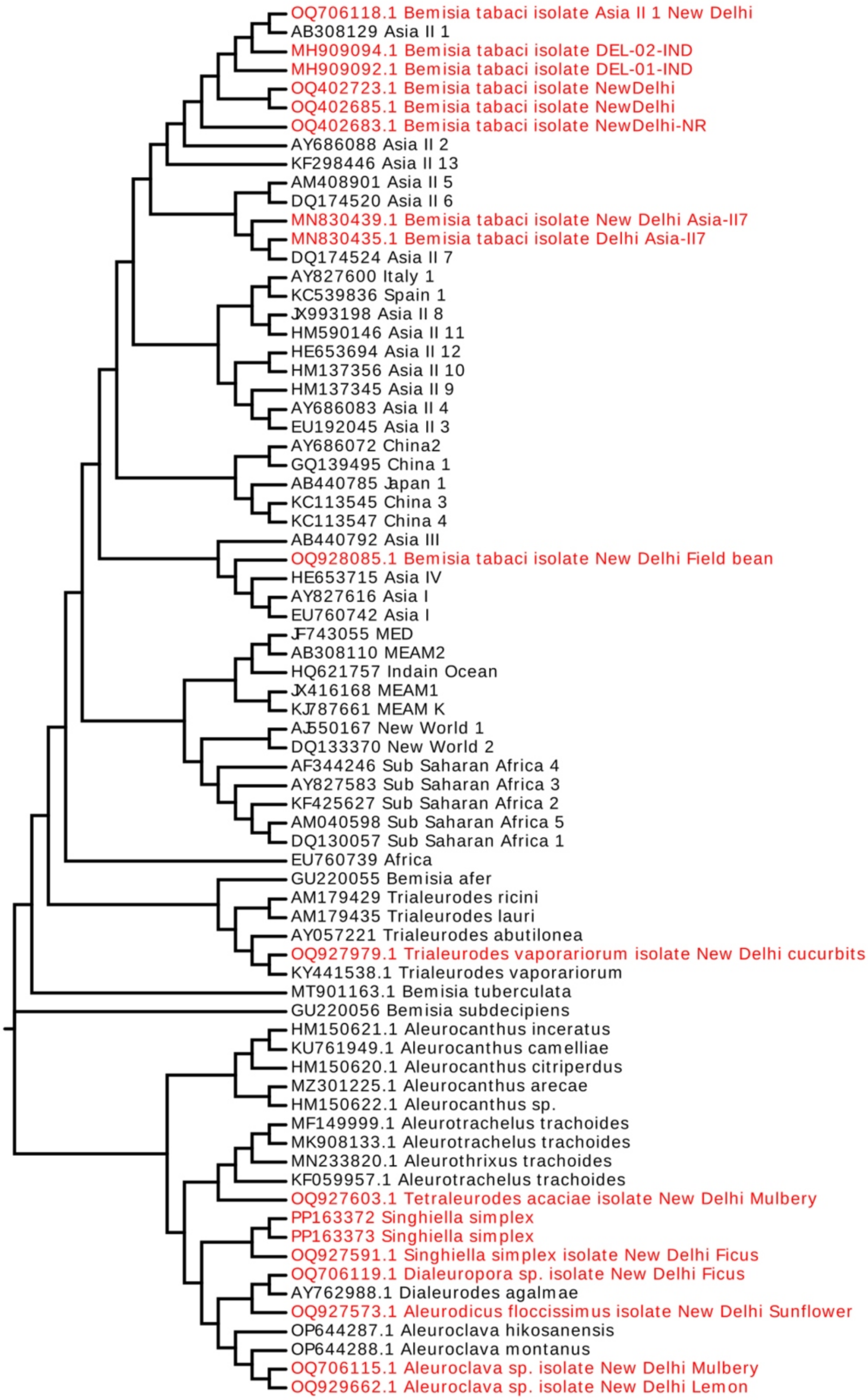

Fig. 2: A phylogenetic tree illustrating the relationships among mtCOI mitotypes of *Bemisia tabaci* and different whitefly species, constructed using the maximum likelihood approach in MEGA 11. Sequences generated in the present study are highlighted in red for clarity. This tree provides a visual representation of the genetic associations, aiding in the understanding of evolutionary patterns and genetic diversity within the studied whitefly populations.

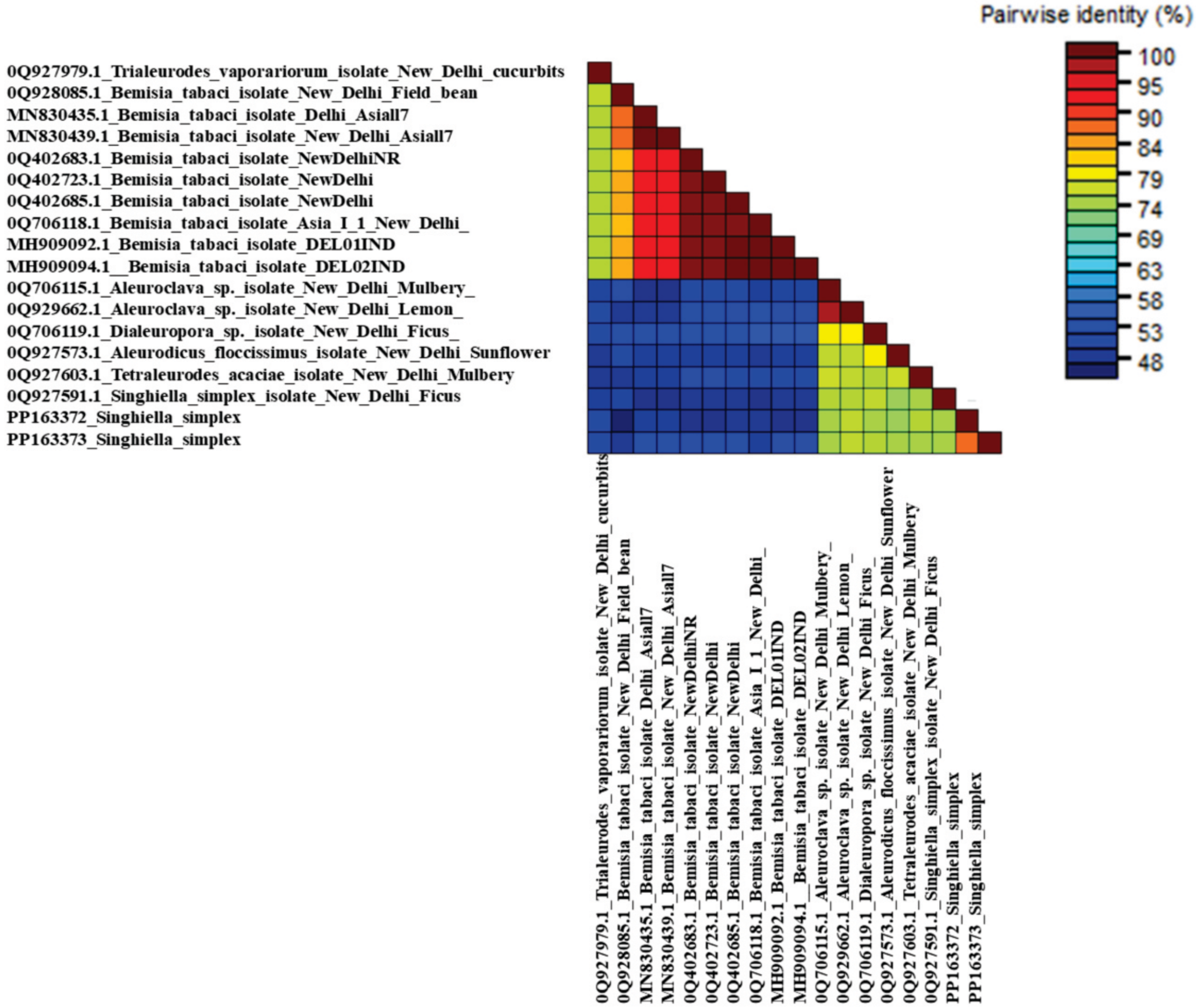

Fig. 3: A color-coded pair-wise identity matrix depicting similarity scores of mitotypes of *Bemisia tabaci* and other whitefly species, generated using the Sequence Demarcation Tool Version 1.2 (SDTv1.2). Each cell in the matrix represents the percentage similarity between two mitotypes, with a color gradient indicating the degree of similarity. The warmer colors (e.g., red) denote higher similarity scores, while cooler colors (e.g., blue) represent lower similarity. This matrix offers a visual representation of the genetic relationships and similarities among different whitefly species, aiding in the assessment of their molecular divergence and evolutionary patterns. Sequences from the present study are included in the analysis to contribute valuable insights into the genetic diversity within *B. tabaci* and its comparison with other whitefly species.

whitefly species, *Aleuroclava* and *Singhella,* were grouped together with the same sequence, and the rest of the four-whitefly genus were grouped separately (Fig. 2). The study findings suggest that the primary whitefly genetic groups of *B. tabaci* in New Delhi are Asia II-1, followed by Asia II-7 and Asia 1. These results align with the findings of Hashmi *et al.* (2018) who previously identified Asia II-1 and Asia II-7 as the predominant genetic groups in Delhi. The host of *B. tabaci* genetic groups reported in this study also aligns with the findings of Ellango *et al.* (2015) and Rehman *et al.* (2021), who reported that *B. tabaci* Asia 1 encompasses a diverse range of over 200 species across various plant families, including Solanaceae (tomato, eggplant, potato, pepper), Malvaceae (okra, cotton), Cucurbitaceae (melon, cucumber, squash), Fabaceae (beans), Brassicaceae (cabbage), and Euphorbiaceae (cassava). On the other hand, Asia II-1 exhibits a preference for Solanaceae, particularly tomato, eggplant and pepper, while also feeding on Malvaceae and Cucurbitaceae (Ashfaq *et al.,* 2014; Firdaus *et al.,* 2013).

Asia II-7 primarily targets Solanaceae and Malvaceae, with a strong inclination towards cotton and okra (Shadmany *et al.,* 2019). These distinct preferences within the *B. tabaci* species highlight their adaptability and the need for targeted management strategies across different crops. Exotic invasive whiteflies

(Hemiptera: Aleyrodidae) have inflicted direct and indirect yield losses on agriculture, horticulture and forestry crops in India over the past 25 years. The invasion began with the establishment of the spiralling whitefly, *A. dispersus* Russell, which invaded and adapted to numerous host plants, including economically significant crops (Sundararaj *et al.,* 2021). In the last five years, seven additional whiteflies have invaded India, originating mainly from the Neotropical region, particularly Central America and the Caribbean. These invasive species, such as the solanum whitefly, rugose spiralling whitefly, nesting whiteflies (*Paraleyrodes bondari* and *P. minei*), legume-feeding whitefly (*T. acaciae*), palm-infesting whitefly (*Aleurotrachelus atratus*) and woolly whitefly (*Aleurothrixus floccosus*) have extensively spread, causing significant agricultural and economic impacts (Sundararaj *et al.,* 2021). Additional genera of whiteflies, namely *T. acaciae, Trialeurodes vaporariorum, Aleuroclava* sp., *Dialeurodes* sp., *S. simplex* and *A. floccissimus* have been documented. *T. acaciae* was recorded in India on subabul, with subsequent infestations observed on orchid trees, tamarind, rain trees and an unidentified pulse crop (Sundararaj and Vimala, 2018). The impact of *T. vaporariorum* extends to vegetables and ornamental species, requiring comprehensive management strategies, as outlined by Paschapur *et al.* (2023).

The shift in damaging patterns, transitioning from greenhouses to open fields, raises significant concerns (Paschapur *et al.,* 2023). *A. floccissimus* primarily infests citrus trees and may also feed on guava, mango, litchi and ornamental plants (Sundararaj *et al*., 2020). However, specific information regarding its presence in India highlights its notable absence, making this study, the study to confirm its presence in the country. Reports on *Aleuroclava,* a potential whitefly species, have been documented by Pushpa and Sundararaj (2010); further research is required to extend the host, extent of damage, and distribution in India. *S. simplex* is considered an Asian whitefly with origin in China, India, and Myanmar. It was first reported by Singh in 1931 from Pusa in India (Singh, 1932). The insect has spread throughout the Mediterranean in less than a decade. *D. citri* is a major citrus tree pest; *Dialeurodes kirkaldyi* predominantly infests cotton and okra; and *Dialeurodes tracheae* is suggested to occur in India, emphasising the need for further research (Mahmoudi *et al.*, 2024; Vasantharaj David and Sundararaj, 1993). Understanding the taxonomy, life cycle, and biological control methods of whiteflies is crucial for effective pest management, potentially averting the extensive use of pesticides in agriculture. Population Genetic research is recommended to examine diversity, distribution and alterations in population structure, providing valuable insights into the dynamics of significant pests like *B. tabaci.*

Understanding the identity of whitefly species is crucial for effective pest management due to variations in their susceptibility to chemicals and differences in physiological attributes. Molecular tools, such as genetic analysis, play a vital role in species identification and exploring genetic diversity within the *B. tabaci* species complex. This study, focus on the genetic diversity of whitefly pests and shed light on variations in susceptibility to insecticides and physiological traits among different genetic groups. Future research should delve into deeper genetic analyses, assess the host range and damage potential of identified species, explore sustainable biological control measures, monitor population dynamics, and foster global collaboration for a more comprehensive understanding of whitefly ecology and management strategies. Addressing these objectives will contribute to the development of resilient and sustainable agricultural practices in the face of evolving pest pressures.

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