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POPULATION DYNAMICS AND MOLECULAR CHARACTERIZATION OF WHITEFLY, *Bemisia tabaci* INFESTING OKRA

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ABSTRACT

Studies were carried out on population dynamics and molecular diversity of okra whitefly at the College Farm, N. M. College of Agriculture, Navsari Agricultural University, Navsari during summer. The infestation of whitefly was commenced from third week after sowing (10th SMW) with 1.2 whiteflies per plant. Whitefly population was significant and positively correlated with maximum temperature. Whereas, it had shown positive and non-significant correlation with minimum temperature, morning relative humidity and wind speed. Correlation between whitefly population and evening relative humidity was negative and non-significant.

Obtained results from the BLAST tool suggested that the Navsari and Surat sequenced showed the highest identity of 100 per cent with *Bemisia tabaci* isolate Devarayapuram DeP cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial complete sequence with accession number OP646480.1, OP601400.1. While, Bharuch sequenced showed the highest identify of 99.49 per cent with *B. tabaci* complete sequence with accession number MN532124.1. The phylogenetic tree showed two main clusters, A cluster and B cluster. Further A12 cluster included NAU-Bharuch mtCOI gene segment cytochrome c oxidase subunit I (COX1) gene accession number PP600771.1. A2 further divided into A21 and A22. In A22 cluster, included NAU-Navsari and NAU-Surat with accession number PP601002.1 and PP600908.1, respectively. Three different whitefly sequences that were obtained from okra crop of three different locations clustered with the cryptic species Asia II 1. The genetic variation of 0.4 per cent between the whitefly samples from Navsari and Surat. While, genetic dissimilarity between the Surat and Bharuch whitefly samples was 13.9 per cent. The genetic difference between the Bharuch and Navsari whitefly samples was 14.4 per cent.

Key words: Okra, whitefly, *Bemisia tabaci*, population dynamics, mtCOI gene, molecular diversity

Introduction

Okra, *Abelmoschus esculentus* (L.) Moench is one of the most important vegetable crop has its own importance, an account of its taste, flavour and nutritional values as human food grown in tropical and sub-tropical parts of the world. Okra is good source of vitamin, calcium, phosphorus, potassium, protein, carbohydrates, fats, minerals, iron, iodine and acids and plays important role in human diet (Bisen *et al.*, 2020). Incidence of insect pests is one of the prime factors in lower production of okra. Among different sucking pests of okra, whitefly,

Bemisia tabaci (Gennadius) causes economic damage to okra by feeding on phloem sap, there by contaminating leaves and fruits with honeydew that causes sooty mold formation (Oliveira *et al.*, 2001). Both nymphs and adults of whitefly suck the cell sap from different parts of the plant causing loss of plant vigour and reduces crop yield (Attique *et al.*, 2003). Besides causing direct damage, it also transmits an economically important viral disease caused by Okra Yellow Vein Mosaic Virus (OYVMV) resulting in significant yield losses especially when it occurs in the early stages of crop growth. Classification

of cryptic species of *B. tabaci* became an over whelming task for the classical taxonomists of morphological traits are poorly understood (Gill and Brown, 2010; Chaubey *et al.*, 2015). Due to these issues, researchers classified whitefly based on traits like genetics, host range, insecticide resistance, behaviour, virus transmission, and interactions with viruses and host plants (Xu *et al.*, 2011; Naveen *et al.*, 2017).

During the past twenty years, many molecular techniques have been employed to identify the *B. tabaci* cryptic species. These techniques include study of 16S rDNA, mitochondrial cytochrome oxidase 1 (COI), and ribosomal internal transcribed spacer 1 sequences. Geographic distribution of *B. tabaci* can altered due to differences in cropping pattern, host plants, and other environmental conditions (Acharya *et al.*, 2020). Among 36 global genetic groups, India has 11 of them (Asia I, Asia I-India, Asia II-1, Asia II-5, Asia II-7, Asia II-8 and Asia II-11, Middle East Asia Minor (MEAM) -1 and Middle East Asia Minor (MEAM) - K genetic groups on different alternate hosts including cotton (Chowda-Reddy *et al.*, 2012; Ellango *et al.*, 2015; Roopa *et al.*, 2015). Despite the fact that okra is farmed in all of India's main states, complete data on whitefly, their distribution and diversity (Genetic groups or putative species) on okra are scarce. The present study primarily aimed to identify the species of *B. tabaci* through analysis of COI nucleotide sequences based on their phylogenetic relationship and geographical distribution in south Gujarat.

Materials and Methods

The Seasonal Incidence of Whitefly in Okra

The present investigation was carried out at B block, College Farm, N.M. College of Agriculture, Navsari Agricultural University, Navsari during summer 2023. All the recommended agronomical practices were followed. To find out the incidence on whitefly in okra (Gujarat Anand Okra-8), 50 plants were randomly selected and three leaves representing top, middle and bottom portion of each tagged plant observation was recorded. Mean pest population was worked out and the data was statistically analyzed. Weather data recorded for study the effect of weather parameters on population dynamics of whitefly and simple correlation coefficients was work out between pest population and various weather

parameters.

Molecular Diversity

The molecular diversity was quantified among different species of whitefly, collected from three okra fields, one farmer field each of Surat and Bharuch district and okra field, College Farm, N. M. College of Agriculture, NAU, Navsari (Table 1). This experiment was carried out in the laboratory of Division of Plant Biotechnology, ASPEE Shakilam Biotechnology Institute, Navsari Agricultural University, Surat, during 2023.

Adult whiteflies and pupae were collected from three major okra-growing locations in South Gujarat, namely Eru (Navsari), Kholvad (Surat), and Tham (Bharuch), during the summer season of 2023. Collected specimens were preserved in 70% ethanol and stored at 4°C until molecular analysis.

Genomic DNA was extracted from individual whiteflies using a modified SDS-Proteinase K protocol, followed by purification with RNase treatment and phenol:chloroform:isoamyl alcohol extraction. DNA quality and concentration were assessed using UV spectrophotometry (A260/A280 ratio) and agarose gel electrophoresis. DNA samples were standardized to 25 ng/μL for downstream analysis.

A partial fragment of the mitochondrial cytochrome c oxidase subunit I (mtCOI) gene was amplified by polymerase chain reaction (PCR) using the universal primers LCO1490 (52 -GGTCAACAAATCATAAAGA TATTGG-32) and HCO2198 (52 -TAAACTTCAGGGT GACCAAAAAATCA-32). PCR products were resolved on 1.2% agarose gel, purified, and subjected to Sanger sequencing.

The obtained nucleotide sequences were compared with reference sequences available in the NCBI GenBank database using the BLAST program for species confirmation. Sequence alignment and phylogenetic analyses were performed using MEGA software to assess the genetic diversity and evolutionary relationships among whitefly populations collected from different okra-growing regions of South Gujarat.

Genetic diversity analysis (Phylogenetic analysis)

The obtained Mt COI sequences from the sequencing of amplification PCR product was further analyzing for

Table 1: Sampling for collection of whitefly from okra growing areas in South Gujarat during summer 2023.

Sr. No.	Name of district	Name of Taluka	Date of collection	Latitude	Longitude	Name of villages	No. of samples collected
1	Navsari	Jalalpore	14/04/2023	20.9195 °N	72.89567 °E	Eru	100
2	Surat	Kamrej	01/07/2023	21.27761 °N	72.96837 °E	Kholvad	100
3	Bharuch	Bharuch	20/05/2023	21.74185 °N	72.95658 °E	Tham	100

phylogenetic analysis by using MEGA (Molecular Genetics analysis) software.

Results and Discussion

The Seasonal Incidence of Whitefly in Okra

The weekly average whitefly per plant was given in and graphically depicted in Fig. 1. The infestation of whitefly was commenced from third week after sowing (10th SMW) with 1.2 whiteflies per plant. Further, the population of whitefly increased gradually and reach its peak in the third week of April *i.e.* 16th SMW (12.9 whiteflies /plant), thereafter the population of whitefly gradually decreased and found 3.1 whiteflies/plant in last week of May. Thus from the result of seasonal incidence, it can be inferred that the whitefly infestation started during March and reached peak level in month of April.

The result of present investigation are closely associated with Ambaliya (2022), indicated that the incidence of whitefly was started from 8th SMW with highest population peak was found during 14th SMW. Dhandge *et al.*, (2018) found that the incidence of whitefly was noticed during 3 week after sowing (10th SMW) and reached peak in 16th SMW. Sahito *et al.*, (2012) reported found that the whitefly population gradually peaked to in 1st week of April. Burade *et al.*, (2019) found that infestation of whitefly commenced in the 10th MW and reached its peak in the 21st SMW. According to Patel *et al.*, (2022), the population of whitefly incidence noticed from 11th SMW and reached its peak in 17th SMW in 2021, while during 2022 it was noticed from 10th SMW and reached its peak in 16th SMW.

Correlation of whitefly with weather parameters

The result presented in Table 2 and graphically depicted in Fig. 2 indicated that the positive and significant correlation was existed between whitefly population and maximum temperature ($r= 0.564$), while non-significant positive correlations were observed with minimum temperature ($r= 0.328$), morning relative humidity ($r=$

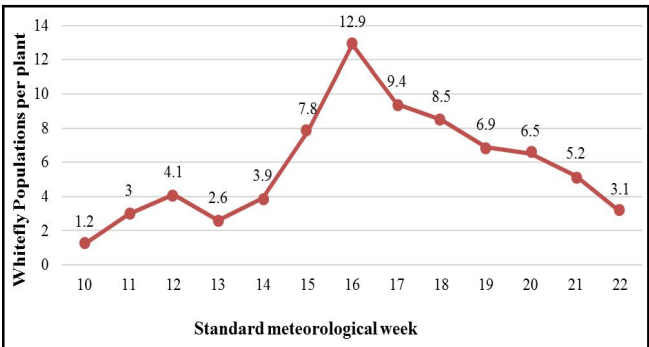


Fig. 1: Population dynamics of whitefly on okra during summer 2023.

Table 2: Correlation coefficient of whitefly with weather parameters.

Weather parameters	Temperature		RH		WS (km/hrs)
	Max	Min.	Morning	Evening	
Whitefly	0.560*	0.334	0.072	-0.022	0.057

RH: Relative humidity (%); WS: Wind speed
* Significant at 5% level of significant

0.081) and wind speed ($r= 0.046$). Correlation between whitefly population and evening relative humidity ($r= -0.031$), was negative non-significant. Thus, the results clearly indicated that incidence of whitefly was directly influenced by maximum temperature.

The present find supported by the Mohanasundaram and Sharma (2011), who found that infestation of whitefly population exhibited negative non-significant correlation with evening relative humidity and morning relative humidity. The positive and significant correlation was found between whitefly and maximum temperature and positive non-significant correlation with minimum temperature and wind speed, whereas non-significant negative correlation with evening relative humidity reported by Ambaliya (2022) and Aravinda *et al.*, (2022). The result obtained in present studies are in close agreement with above worker, thus confirm the present findings.

Molecular Diversity

Isolation of DNA:

Adult whitefly genomic DNA was isolated from whitefly by protocol given by Kale (2013) with some modification. After DNA extraction, the DNA concentration was determined using Nano-drop to ascertain its purity and quantity. Quality of DNA was

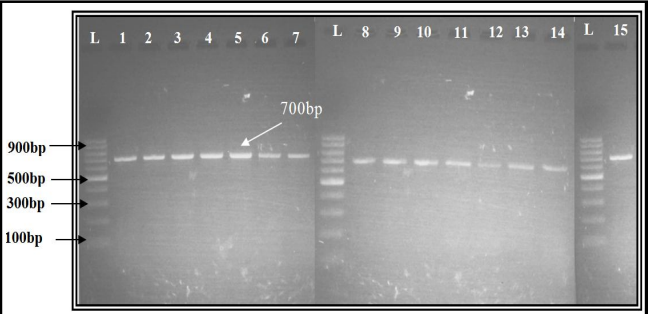


Photo 1: PCR amplification of the mtCOI gene cytochrome c oxidase subunit I (COX1) of whitefly collected from Navsari, Surat and Bharuch.

L- 100 bp DNA ladder		
1. Navsari 1	2. Navsari 2	3. Navsari 3
4. Navsari	5. Navsari 5	6. Surat 1
7. Surat 2	8. Surat 3	9. Surat 4
10. Surat 5	11. Bharuch 1	12. Bharuch 2
13. Bharuch 3	14. Bharuch 4	15. Bharuch 5

check by running on 0.8% Agarose gel.

PCR Amplification of mtCOI Component of Whitefly

In order to the amplify nucleotide sequence of whitefly, universal primers were used to amplify the mtCOI region of the whitefly (Folmer *et al.*, 1994). The amplicon of mtCOI genes was confirmed by electrophoresis and ~ 700 bp bands were found to confirm the presence of mtCOI gene in all whitefly samples. A DNA fragment of mtCOI was amplified with LCO and HCO primers of the whitefly at ~ 700bp amplicon (Photo 1).

Sequencing of mtCOI gene cytochrome c oxidase subunit I (COX1) Component of Whitefly

The results revealed that the sequenced mtCOI had a 610 bp (Navsari), 559 bp (Surat), 590 bp (Bharuch) long nucleotide sequence. The results of the BLAST (<http://www.ncbi.nih.gov/BLAST>) search was performed to identified sequence homology, clearly demonstrated that the mtCOI gene sequence of whitefly matched range 100-97.97 per cent with previously reported isolates of whitefly from different geographical locations. Further, the sequence has been submitted to GeneBank, NCBI, Bethesda Maryland, USA using the Bankit submission tool of NCBI (<https://www.ncbi.nlm.nih.gov>). Accession number PP601002.1 (Navsari), PP600908.1 (Surat), PP600771.1 (Bharuch) was given to the sequence by Gene Bank in the public domain.

Phylogenetic Analysis of the Sequence and Comparison with other *B. tabaci* Multiple Alignment and Similarity Index

Obtained results from the BLAST tool suggested that mtCOI gene of whitefly of locations, Navsari and Surat sequenced showed the highest identity of 100 per cent with *B. tabaci* isolate Devarayapuram DeP cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial complete sequence with accession number OP646480.1, OP601400.1 Followed by 99.81 per cent

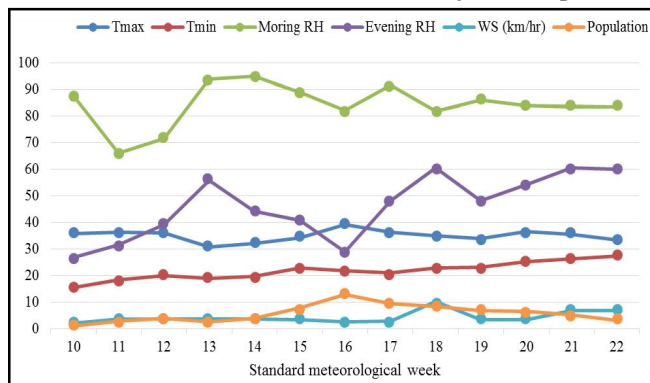


Fig. 2: Population dynamics of whitefly on okra with weather parameters during summer 2023.

sequence identity with accession number MK333460.1, MW193997.1, MW193995.1, MW193991.1, KF298438.1, while 99.62 per cent sequence identity with accession number MW194004.1. While, Bharuch sequenced showed the highest identify of 99.49 per cent with *B. tabaci* complete sequence with accession number MN532124.1 followed by 99.32 per cent sequence identify with HM185755.1, JN410736.1(99.15%), JQ995273.1 (98.64%), MK000727.1 (98.31%), KJ677037.1(98.31%), KJ709450.1 (98.46%), JN703442.1 (98.31%), KU666425.1 (97.97%), JN859139.1 (97.97%).

Phylogenetic analysis of mtCOI gene segment of cytochrome c oxidase subunit I (COX1) of *B. tabaci* with other *B. tabaci*

The mtCOI gene sequences from the present study was used for homology study and phylogenetic analysis with previously reported 18 different mtCOI gene sequences downloaded from NCBI. Distance matrix was performed for all 21 mtCOI gene sequences by using BioNJ algorithms.

Multiple sequence alignments of *B. tabaci* isolate NAU-Navsari, NAU-Surat and NAU-Bharuch mtCOI gene segment cytochrome c oxidase subunit I (COX1) gene, accession number PP601002.1 (Navsari), PP600908.1(Surat), PP600771.1 (Bharuch) were carried out by using CLUSTALW (2) programme (<https://www.ebi.ac.uk/Tools/msa/clustalw2/>) to find the conserved sequences among all the mtCOI gene of *B. tabaci* sequences. Further, a phylogenetic tree was constructed by the Neighbour-Joining method with 1,000 bootstrap replications. The phylogenetic tree built using the mtCOI gene of *B. tabaci* isolate NAU-Navsari, NAU-Surat and NAU-Bharuch mtCOI gene of cytochrome c oxidase subunit I (COX1) showed two main

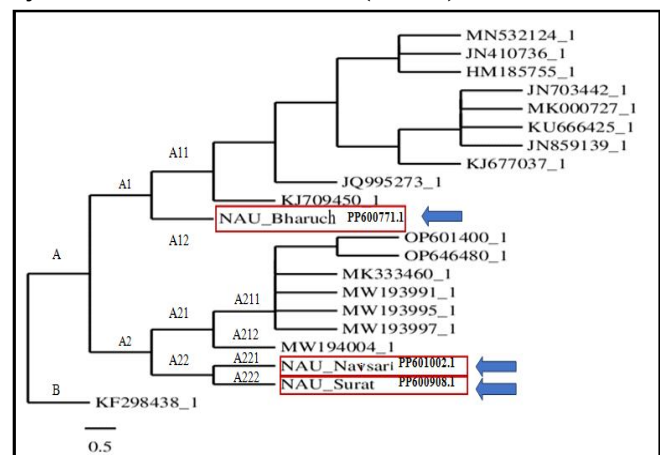


Fig. 3: Phylogenetic tree sequences of sequences of mtCOI gene segment cytochrome c oxidase subunit I (COX1) gene of whitefly (NAU_Navsari, NAU_Surat and NAU_Bharuch) and previously reported whitefly.

clusters (Fig. 3). Further cluster A, divided into two subdivisions as cluster A11 and A12. Among A12 included NAU-Bharuch mtCOI gene segment cytochrome c oxidase subunit I (COX1) gene accession number PP600771.1 and A11 included KJ709450.1, JQ995273.1, KJ677037.1, JN859139.1, KU66425.1, MK000727.1, JN703442.1, HM185755.1, JN410736.1, MN532124.1, followed by cluster A2. A2 further divided into A21 and A22. A22 included NAU-Navsari and NAU-Surat with accession number PP601002.1 and PP600908.1, respectively, and A21 included MW194004.1, MW193997.1, MW193995.1, MW193991.1, MK333460.1, OP646480.1, OP601400.1 while, cluster B included KF298438.1.

Three different whitefly sequences that were obtained from okra crop of three different locations (Navsari, Surat, and Bharuch) clustered with the Asia II 1. These specimens were collected from 3 locations (Navsari, Surat and Bharuch) of South Gujarat. Thus, all the sequences characterized in this study were assigned to the one cryptic species (Asia II 1) identified by Dinsdale *et al.*, (2010). The present findings are supported by Ellango *et al.*, (2015) who reported Asia II 1 from Anand and Surat. 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 (Prasanna *et al.*, 2015). Ahmed *et al.*, (2011) found Asia 1, Asia II 1 and Middle East-Asia Minor in Punjab. Ashfaq *et al.*, (2014) demonstrated the existence of Asia II 1, Middle East-Asia Minor 1, Asia 1, Asia II 5, Asia II 7, and a new lineage known as “Pakistan” were present. Islam *et al.*, (2018) revealed that the presence of five different cryptic species in Pakistan namely Asia II-1, Asia II-5, Asia II-7, Asia II-8 and MEAM-1.

The dissimilarity matrix table ndix IX), constructed using sequences of the mtCOI gene segment (cytochrome c oxidase subunit I, COX1 gene), reveals varying degrees of genetic differentiation. Specifically, the genetic variation of 0.4 per cent between the whitefly samples from Navsari and Surat. While, genetic dissimilarity between the Surat and Bharuch whitefly samples was 13.9 per cent. The genetic difference between the Bharuch and Navsari whitefly samples was 14.4 per cent.

Using mtCOI gene primers LCO and HCO of the whitefly, genomic DNA fragment of the mtCOI gene was amplified at ~ 700 bp in all the different locations of the whitefly (Navsari 1, Navsari 2, Navsari 3, Navsari 4, Navsari 5, Surat 1, Surat 2, Surat 3, Surat 4, Surat 5, Bharuch 1, Bharuch 2, Bharuch 3, Bharuch 4, Bharuch 5). A nucleotide sequence measuring 610 bp (Navsari),

559 bp (Surat), and 590 bp (Bharuch) was obtained. Further the sequence has been submitted to Genebank, NCBI, Bethesda, Maryland, UAS. Accession number Navsari (PP601002.1), Surat (PP600908.1) and Bharuch (PP600771.1) were given to the sequence by Genebank.

Obtained results from the BLAST tool suggested that the Navsari and Surat sequenced showed the highest identity of 100 per cent with *Bemisia tabaci* isolate DevarayapuramDeP cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial complete sequence with accession number OP646480.1, OP601400.1. While, Bharuch sequenced showed the highest identify of 99.49 per cent with *B. tabaci* complete sequence with accession number MN532124.1.

The phylogenetic tree built using the mtCOI gene of *B. tabaci* isolate NAU-Navsari, NAU-Surat and NAU-Bharuch mtCOI gene of cytochrome c oxidase subunit I (COX1) showed two main clusters, Further cluster A divided into two subdivisions as cluster A11 and A12. Among A12 included NAU-Bharuch mtCOI gene segment cytochrome c oxidase subunit I (COX1) gene accession number PP600771.1 and A11 included KJ709450.1, JQ995273.1, KJ677037.1, JN859139.1, KU66425.1, MK000727.1, JN703442.1, HM185755.1, JN410736.1, MN532124.1, followed by

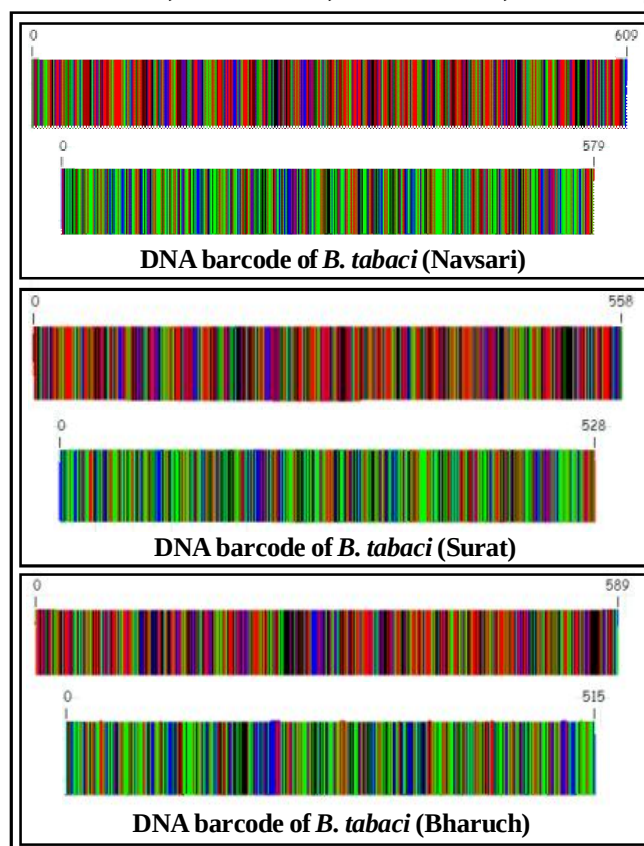


Fig. 4: DNA barcodes of whitefly collected from Navsari, Surat and Bharuch.

cluster A2. A2 further divided into A21 and A22. A22 included NAU-Navsari and NAU-Surat with accession number PP601002.1 and PP600908.1, respectively, and A21 included MW194004.1, MW193997.1, MW193995.1, MW193991.1, MK333460.1, OP646480.1, OP601400.1 while, cluster B included KF298438.1.

Three different whitefly sequences that were obtained from okra crop of three different locations (Navsari, Surat and Bharuch) clustered with the cryptic species Asia II 1. Thus all the sequences characterized in this study were assigned to the one cryptic species Asia II 1. The genetic variation of 0.4 per cent between the whitefly samples from Navsari and Surat. While, genetic dissimilarity between the Surat and Bharuch whitefly samples was 13.9 per cent. The genetic difference between the Bharuch and Navsari whitefly samples was 14.4 per cent.

DNA Barcode Submission to BOLD

Specimen-level metadata, including taxonomy, sequences, trace files and images of *B. tabaci* from three locations of South Gujarat, were submitted to the Barcode of Life Data System (BOLD). Unique process IDs were assigned and COXI and COXII barcodes generated for reliable identification and comparative analysis (Fig. 4). This submission enhances global accessibility and supports future studies on genetic diversity, biogeography, and pest diagnostics.

Conclusion

The infestation of whitefly was commenced from 3rd week after sowing (10th SMW) with maximum (12.9/3 leaves) in 9th week after sowing (16th SMW). Whitefly population was significant and positively correlated with maximum temperature. Whereas, it had shown positive and non-significant correlation with minimum temperature, morning relative humidity and wind speed, while exhibited negative and non-significant correlation with evening relative humidity.

The survey and analyses performed here suggest distinct genetic structuring among the whitefly populations across different locations of South Gujarat and provide a picture of species composition and diversity within whitefly complex in okra. The information generated here could be useful for monitoring future patterns of whitefly population diversity and abundance. Although the exact reasons for the observed patterns of diversity are not known, we believe that the information uncovered here has potential to aid developing targeted management strategies against whitefly and whitefly transmitted viral diseases in okra.

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