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DNA BARCODING AND MOLECULAR CHARACTERIZATION OF SPIDER MITE (*Tetranychus urticae*: Tetranychidae) INFESTING OKRA USING MITOCHONDRIAL COI GENE

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ABSTRACT

Spider mites are serious pests of okra and accurate species identification is essential for effective pest management. The present study aimed to characterized spider mite populations collected from seven major okra-growing regions of Gujarat using mitochondrial cytochrome c oxidase subunit I (*COI I*) and (*COI II*) gene sequences. Genomic DNA was isolated using a modified CTAB method and the target gene regions were successfully amplified through PCR followed by Sanger sequencing. BLASTn analysis confirmed that specimen collected from the Narmada region showed 98.91 per cent sequence similarity with *Tetranychus urticae*, while specimen collected from Navsari, Anand, Surat, Tapi, Valsad and Bharuch ii showed (98-99 per cent) sequence similarity with *Tetranychus macfarlanei* and *Tetranychus malaysiensis*. *T. malaysiensis* has been reported as a junior synonym of *T. macfarlanei*. Nucleotide composition revealed an AT-rich mitochondrial genome. Phylogenetic analysis using the Maximum Likelihood method and Kimura 2-parameter model grouped most Gujarat populations into a single clade with slight divergence in the Narmada and Navsari populations. Pairwise genetic distance analysis indicated low genetic variation among the studied populations. The generated sequences were successfully submitted to GenBank and the Barcode of Life Data System (BOLD). The results demonstrated that mitochondrial *COI* markers are reliable tools for DNA barcoding, species identification and assessment of genetic diversity in spider mites associated with okra.

Key words: *Tetranychus urticae*, DNA barcoding, *COI* gene, PCR, BLASTn, phylogenetic analysis & BOLD.

Introduction

Okra (*Abelmoschus esculentus* L.) is an important vegetable crop cultivated in tropical and subtropical regions for its nutritional and economic value. In India, okra is grown extensively however; its productivity is affected by several insect and mite pests. Among them, spider mites (*Tetranychus* spp.) are serious pests causing yellow speckling, chlorosis, leaf curling, drying of leaves and significant yield losses (Jeppson *et al.*, 1975; Zhang, 2003).

Identification of spider mite species based solely on morphological characters is often difficult because of their

minute size and overlapping taxonomic characters. Therefore, molecular approaches such as DNA barcoding have become reliable tools for accurate species identification and genetic characterization (Hebert *et al.*, 2004). Among the available molecular markers, mitochondrial cytochrome c oxidase subunit I (*COI I*) and cytochrome c oxidase subunit II (*COI II*) genes are widely used due to their high amplification efficiency, maternal inheritance and low intraspecific variation (Folmer *et al.*, 1994; Simon *et al.*, 1994).

Sequence comparison using BLAST, phylogenetic analysis and DNA barcode databases such as GenBank

and the Barcode of Life Data System (BOLD) have greatly improved the accuracy of species identification and the assessment of genetic diversity (Altschul *et al.*, 1990; Ratnasingham and Hebert, 2007). Information on the molecular diversity of spider mite populations associated with okra in Gujarat is limited. Therefore, the present study was undertaken to characterized spider mite populations collected from different agro-climatic regions of Gujarat using mitochondrial *COI* I and *COI* II gene sequences. The generated DNA barcode data will contribute to accurate species identification, genetic diversity analysis and future pest management strategies.

Materials and Method

Sample Collection and Preservation

During 2024-2025, the samples of okra leaves infested with spider mites were collected from different farmer field location Navsari, Anand, Surat, Tapi, Valsad, Narmada and Bharuch. Adult female mites were collected from infested okra leaves and maintained as single gravid female isolines under laboratory conditions. Representative specimens were preserved in 70 per cent ethanol and stored at -20°C until molecular analysis. Molecular work was carried out at the Department of Plant Molecular Biology and Biotechnology, NMCA, NAU, Navsari, to investigate intra-species genetic variation across different agro climatic zones.

Genomic DNA Isolation

Total genomic DNA was extracted from five to ten female mites obtained from an isolate following the modified CTAB method described by (Rogers and Benedich, 1994; Kadu, 2019). The quality of isolated DNA was verified through agarose gel electrophoresis, which showed clear and intact bands without contamination. Additionally, spectrophotometric analysis recorded an A_{260}/A_{280} absorbance ratio ~1.8 indicating good quality and pure DNA.

PCR Amplification and Purification

Mitochondrial *COI* I and *COI* II regions were amplified using gene-specific primers (Table 1). The *COI* I region was amplified using *COI* F and *COI* R primers, whereas the *COI* II region was amplified using LepF1 and LepR1 primers, recommended by (Simon *et al.*, 1994; Sheffield *et al.*, 2009). The PCR reaction mixture was prepared in a total volume of 60.0 µl, consisting of PCR Buffer 6.0 µl, dNTP 1.5 µl, Taq polymerase 1.5 µl, 3 µl forward primer (10 pmol), 3.0 µl of reverse primer (10 pmol), 6.0 µl of DNA template (containing 50 ng of DNA) and 39.0 µl of nuclease-free water. The cycling program consisted of initial denaturation at 94°C, followed by 35 cycles of denaturation for 30 seconds at 94 °C, annealing for 45 seconds at 58 °C and extension for 45 seconds at 72 °C, with a final extension of 3 min at 72 °C. Amplicons

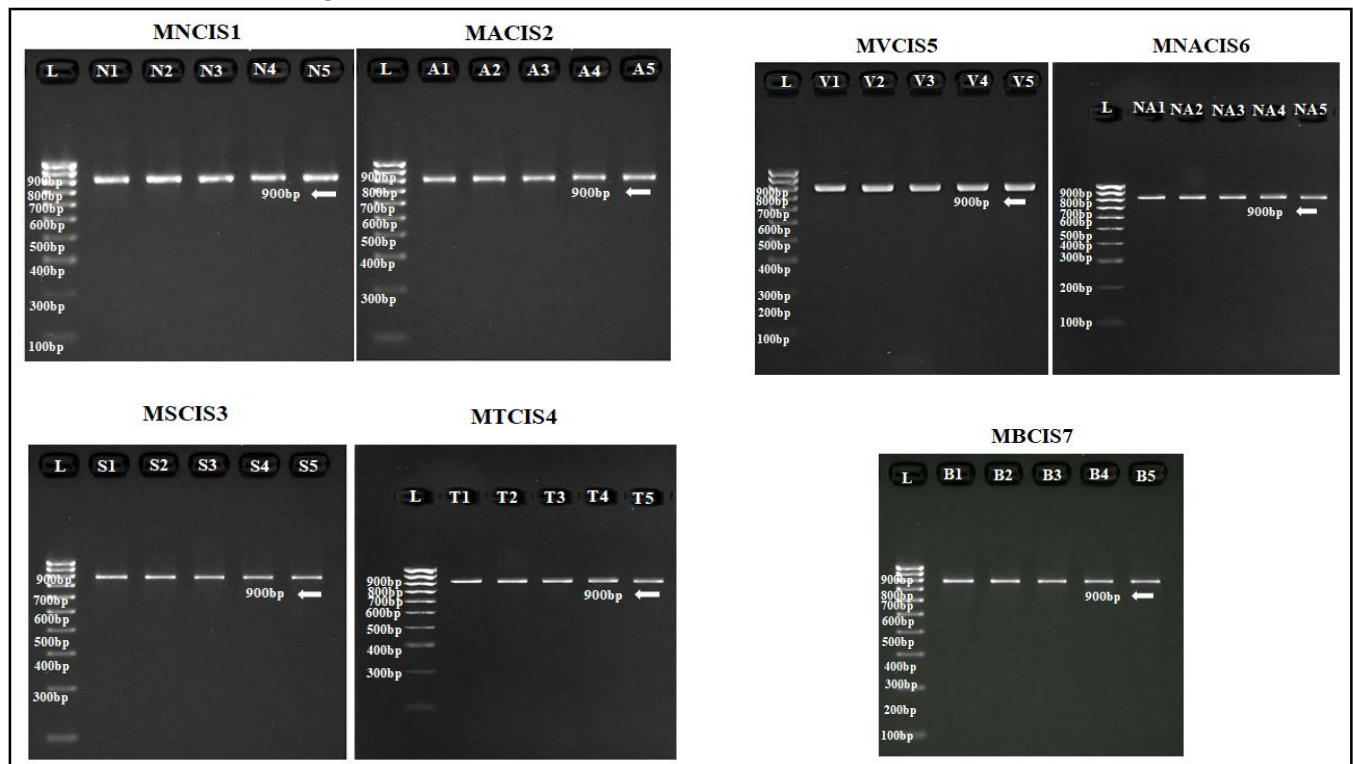


Fig. 1: PCR amplification of *COI* I gene of spider mite collected from Navsari, Anand, Surat, Tapi, Valsad, Narmada and Bharuch location. Representative sample shown: Ladder (L: 100bp); Navsari (N1 to N5); Anand (A1 to A5); Surat (S1 to S5); Tapi (T1 to T5); Valsad (V1 to V5); Narmada (NA1 to NA5) and Bharuch (B1 to B5)

Table 1: Details of primers used for molecular study.

Sr. no.	Locus	Primer Direction	Sequences 5'-3'	Annealing temp. (°C)	References
1	<i>COI</i> F	Forward	GGAGGATTGGAATGATTAGTTCC	58°C	Simon <i>et al.</i> , 1994
2	<i>COI</i> R	Reverse	GATAAAACGTAATGAAAATGAGCTAC		
3	LepF1	Forward	ATTCAACCAATCATAAAGATATTGG	58°C	Sheffield <i>et al.</i> , 2009
4	LepR1	Reverse	TAAACTTCTGGATGTCCAAAAAATCA		
5	<i>COI</i> 101F	Forward	TTCGAGCTGAATTAGGGACTC	58°C	Nagoshi <i>et al.</i> , 2017
6	<i>COI</i> 911R	Reverse	GATGTAAAATATGCTCGTGT		

were confirmed on 2 per cent agarose gels stained with ethidium bromide. PCR products were purified via ethanol-sodium acetate precipitation, resuspended in Hi-Di™ formamide and sequenced by the Sanger method. High-quality reads were trimmed, aligned and used for molecular analysis.

DNA Sequence Analysis

COI I and *COI* II gene were assembled into consensus sequences using Sequencher v5.3, screened in MEGA 12 and cleaned in BioEdit v7. Validated sequences were submitted to NCBI GenBank with accession numbers assigned, and identities confirmed via BLASTn (98% similarity). Barcode gap, nucleotide composition, and genetic divergence were analyzed from ClustalW alignments. Phylogenetic relationships were inferred using Maximum Likelihood (MEGA 12, 1000 bootstraps) and divergence visualized through pairwise distance heat

maps.

Submission to the Barcode of Life Data System (BOLD)

Information related to spider mite specimens, including specimen metadata, images, sequence data and trace files was deposited into the Barcode of Life Data System (BOLD) an online molecular platform designed for researchers and students to access and contribute DNA barcode data (Ratnasingham and Hebert, 2007). The BOLD platform serves as an important resource for species identification, biodiversity studies and molecular taxonomy.

Result and Discussion

Survey and Sample Collection

Survey was conducted during 2025-2026 to collect spider mite specimens infesting okra from seven major

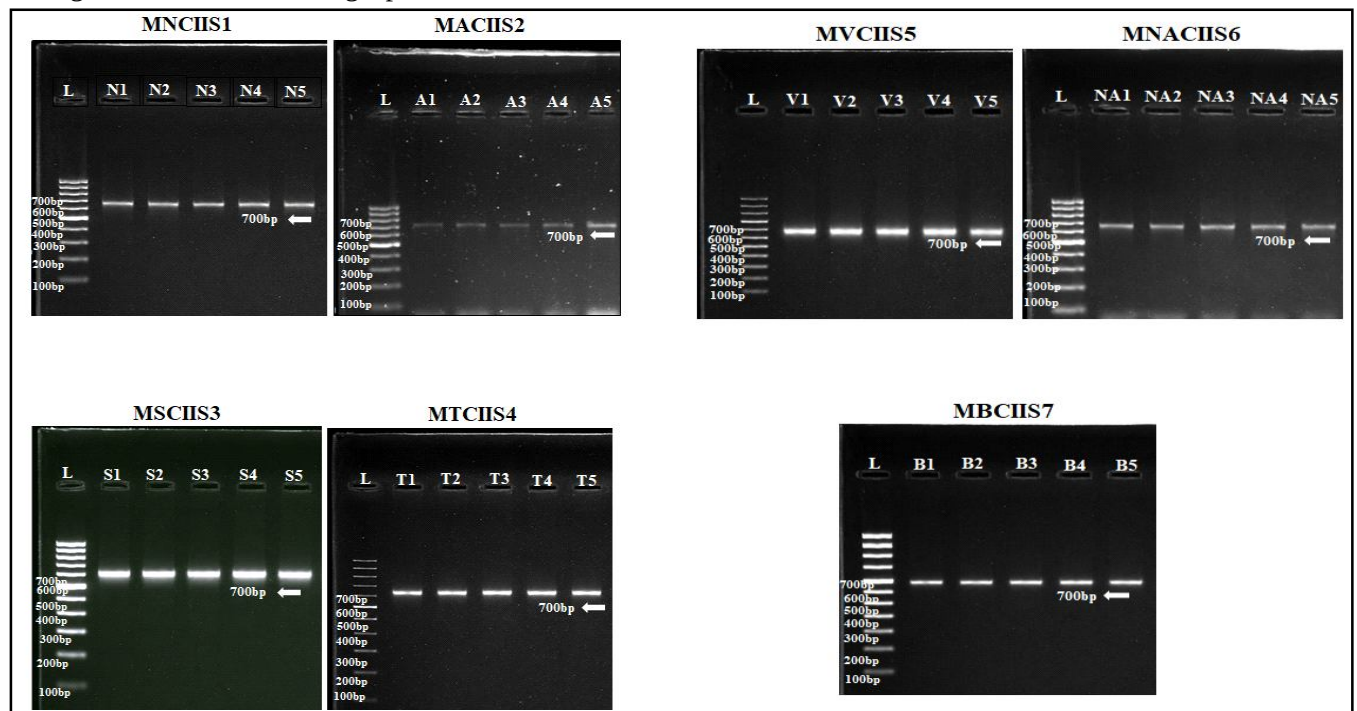


Fig. 2: PCR amplification of *COI* II gene of spider mite collected from Navsari, Anand, Surat, Tapi, Valsad, Narmada and Bharuch location. Representative sample shown: Ladder (L: 100bp); Navsari (N1 to N5); Anand (A1 to A5); Surat (S1 to S5); Tapi (T1 to T5); Valsad (V1 to V5); Narmada (NA1 to NA5) and Bharuch (B1 to B5).

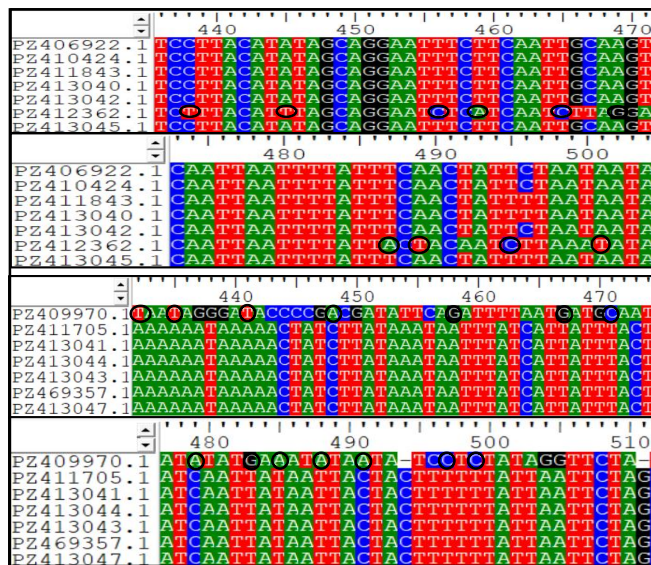
Table 2: Accession numbers of *COI* I and *COI* II gene sequences of spider mite submitted to NCBI GenBank.

Sr. no.	Sample ID	Gene	Accession number
1	MNCIS1	<i>COI</i> I	PZ406922.1
2	MACIS2	<i>COI</i> I	PZ410424.1
3	MSCIS3	<i>COI</i> I	PZ411843.1
4	MTCIS4	<i>COI</i> I	PZ413040.1
5	MVCIS5	<i>COI</i> I	PZ413042.1
6	MNACIS6	<i>COI</i> I	PZ412362.1
7	MBCIS7	<i>COI</i> I	PZ413045.1
8	MNCIIS1	<i>COI</i> II	PZ409970.1
9	MACIIS2	<i>COI</i> II	PZ411705.1
10	MSCIIS3	<i>COI</i> II	PZ413041.1
11	MTCIIS4	<i>COI</i> II	PZ413044.1
12	MVCIIS5	<i>COI</i> II	PZ413043.1
13	MNACIIS6	<i>COI</i> II	PZ469357.1
14	MBCIIS7	<i>COI</i> II	PZ413047.1

agro-climatic regions of Gujarat, namely Navsari, Anand, Surat, Tapi, Valsad, Narmada and Bharuch. Live adult female mites were collected from infested leaves and maintained as single female isolines for molecular studies. Representative specimens from each location were used for genomic DNA extraction and subsequent DNA barcoding analyses. Similar study was conducted by Pokle and Shukla (2015) who reported eight different species belonging to the family Tetranychidae from various crops at Navsari Agricultural University, Gujarat.

Genomic DNA Isolation and DNA Quality Assessment

High-quality genomic DNA was successfully isolated from individual spider mite specimens using the modified CTAB protocol (Rogers and Benedich, 1994; Kadu,

**Fig. 3:** SNP variation for Barcode gap analysis of *COI* I and *COI* II sequence of spider mite individuals collected from different agro-climatic zone of Gujarat.**Table 3:** Percentage composition of AT and GC nucleotides in partial *COI* I and *COI* II gene sequences of spider mite.

Sr. No.	Accession number	Percentage composition of different nucleotide bases					
		T	C	A	G	A+T	G+C
1	PZ406922.1	40.48	12.87	34.87	11.77	75.35	24.64
2	PZ410424.1	40.90	12.84	34.09	12.15	74.99	24.99
3	PZ411843.1	42.69	13.93	32.07	11.28	74.76	25.21
4	PZ413040.1	42.69	13.93	32.07	11.28	74.76	25.21
5	PZ413042.1	40.43	12.85	34.94	11.75	75.37	24.60
6	PZ412362.1	40.90	14.50	31.60	12.98	72.50	27.48
7	PZ413045.1	42.69	13.93	32.07	11.28	74.76	25.21
8	PZ409970.1	41.96	11.21	35.6	11.21	77.56	22.42
9	PZ411705.1	41.48	14.83	33.05	10.62	74.53	25.45
10	PZ413041.1	41.48	14.83	33.05	10.62	74.53	25.45
11	PZ413044.1	41.48	14.83	33.05	10.62	74.53	25.45
12	PZ413043.1	41.48	14.83	33.05	10.62	74.53	25.45
13	PZ469357.1	41.31	14.83	33.38	10.45	74.69	25.28
14	PV590065.1	39.87	14.68	30.91	14.55	70.78	29.22
	Average	41.42	13.92	33.12	11.51	74.55	25.43

2019). The extracted DNA exhibited A_{260}/A_{280} absorbance ratios ranging from 1.80 to 1.85, indicating minimal protein contamination and good DNA quality for downstream molecular analyses. The high purity of genomic DNA ensured efficient PCR amplification and reliable sequencing results. Similar observations have been reported by (Navajas *et al.*, 1996a and Ros and Breeuwer (2007) who demonstrated that high-quality DNA is essential for accurate molecular identification of tetranychid mites.

PCR Amplification and Purification

PCR amplification of the mitochondrial *COI* I and

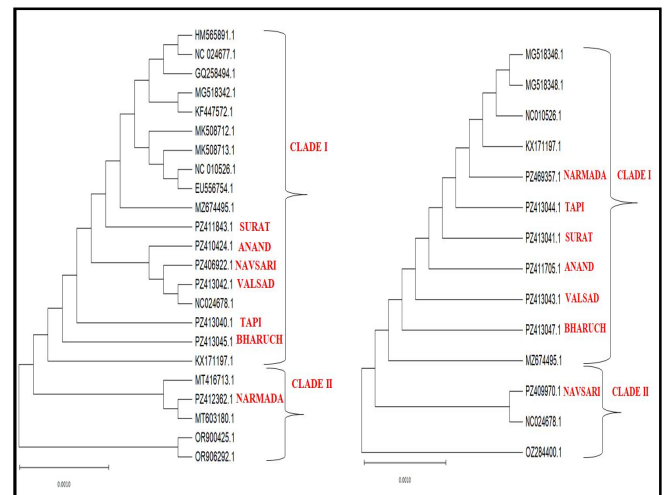
**Fig. 4:** Rooted Neighbor-Joining (NJ) tree illustrating the Phylogenetic clustering of spider mites populations based on partial mitochondrial *COI* I and *COI* II gene sequences using Kimura-2-Parameter (K2P) distances.

Table 4: Genetic distance matrix of different spider mite individuals based on partial mitochondrial (*COII*) gene sequences.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
A																							
B	0.00																						
C	0.07	0.07																					
D	0.07	0.07	0.00																				
E	0.00	0.00	0.07	0.07																			
F	0.39	0.39	0.39	0.39	0.39																		
G	0.07	0.07	0.00	0.00	0.07	0.39																	
H	0.00	0.00	0.07	0.02	0.00	0.39	0.07																
I	0.89	0.94	0.82	0.82	0.89	0.8	0.82	1.46															
J	0.89	0.94	0.82	0.82	0.89	0.87	0.82	1.46	0.00														
K	0.14	0.13	0.15	0.15	0.14	0.39	0.15	1.02	1.24	1.24													
L	0.13	0.13	0.17	0.17	0.13	0.39	0.17	0.13	0.91	0.91	0.08												
M	0.13	0.13	0.16	0.16	0.13	0.41	0.16	0.13	0.91	0.91	0.08	0.05											
N	0.13	0.13	0.17	0.17	0.13	0.42	0.17	0.14	0.92	0.9	0.09	0.08	0.03										
O	0.13	0.13	0.17	0.17	0.13	0.41	0.17	0.13	0.91	0.91	0.08	0.06	0.01	0.00									
P	0.06	0.07	0.01	0.01	0.06	0.42	0.01	0.06	0.87	0.87	0.16	0.17	0.16	0.18	0.19								
Q	0.13	0.13	0.17	0.17	0.13	0.42	0.17	0.98	1.24	1.24	0.26	0.1	0.11	0.12	0.10	0.16							
R	0.14	0.13	0.16	0.16	0.14	0.41	0.16	0.14	0.91	0.91	0.08	0.05	0.00	0.01	0.01	0.16	0.11						
S	0.14	0.13	0.18	0.18	0.14	0.40	0.18	1.02	1.27	1.27	0.27	0.02	0.06	0.07	0.05	0.18	0.30	0.06					
T	0.14	0.13	0.18	0.18	0.14	0.40	0.18	1.03	1.27	1.27	0.27	0.02	0.06	0.07	0.05	0.18	0.3	0.06	0.00				
U	0.37	0.37	0.37	0.37	0.37	0.00	0.37	0.37	0.95	0.95	0.36	0.36	0.39	0.39	0.39	0.4	0.4	0.38	0.38	0.38			
V	0.43	0.42	0.45	0.45	0.43	0.00	0.45	0.43	0.97	0.97	0.43	0.42	0.45	0.48	0.44	0.45	0.45	0.44	0.44	0.44	0.00		
W	0.07	0.07	0.00	0.00	0.07	0.39	0.00	0.07	0.82	0.82	0.15	0.17	0.16	0.17	0.17	0.00	0.17	0.16	0.18	0.18	0.37	0.45	

A = PZ406922.1, B = PZ410424.1, C = PZ411843.1, D = PZ413040.1, E = PZ413042.1, F = PZ412362.1, G = PZ413045.1, H = NC024678.1, I = OR900425.1, J = OR906292.1, K = MG518342.1, L = MK508713.1, M = MK508712.1, N = GQ258494.1, O = HM565891.1, P = MZ674495.1, Q = NC024677.1, R = KF447572.1, S = NC010526.1, T = EU556754.1, U = MT416713.1, V = MT603180.1, W = KX171197.1

COI II gene regions using species-specific primers produced clear and reproducible amplicons of approximately 900 bp and 700 bp, respectively (Fig. 1 and Fig. 2). Successful amplification was obtained from all seven geographical isolates, indicating the suitability of the selected primers for DNA barcoding of spider mite. The amplified products showed distinct bands without non-specific amplification, confirming the specificity and efficiency of the PCR protocol. Comparable amplification of mitochondrial *COI* regions was reported by (Folmer *et al.*, 1994; Hebert *et al.*, 2003) who recognized the *COI* gene as the universal barcode marker for animal species identification. The purified PCR products were subjected to unidirectional Sanger sequencing. High-quality forward sequence was obtained for all amplified samples, allowing accurate sequence assembly and editing.

Analysis of Sequence Homology using BLASTn

BLASTn analysis of the *COI* I and *COI* II sequences confirmed that all spider mite isolates belonged to the genus *Tetranychus*. The specimen collected from the Narmada region showed 98.91 per cent sequence similarity with *Tetranychus urticae*, while specimen

collected from Navsari, Anand, Surat, Tapi, Valsad and Bharuch showed (98 to 99 per cent) sequence similarity with *Tetranychus macfarlanei* and *Tetranychus malaysiensis*.

T. malaysiensis has been reported as a junior synonym of *T. macfarlanei* (Zeity *et al.*, 2017; Baker and Pritchard, 1960). These findings confirmed the effectiveness of mitochondrial *COI* markers for accurate species identification and supported the reliability of DNA barcoding in differentiating closely related *Tetranychus* species. Similar results were reported by (Ros and Breeuwer 2007 and Hebert *et al.*, 2003).

GenBank Sequence Submission

DNA sequences of spider mite generated from the *COI* I and *COI* II genes of specimens collected from seven different locations were deposited in the NCBI GenBank database. A total 14 sequences were submitted to GenBank where each was assigned a unique accession number. The accession numbers presented in (Table 2) provide unique and permanent identifiers for each sequence, thereby ensuring data reproducibility and supporting future investigations in taxonomy, population

Table 5: Genetic distance matrix of different spider mite individuals based on partial mitochondrial (*COI* II) gene sequences.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
A														
B	0.75													
C	0.75	0.00												
D	0.75	0.00	0.00											
E	0.75	0.00	0.00	0.00										
F	0.74	0.00	0.003	0.003	0.003									
G	0.75	0.00	0.00	0.00	0.00	0.003								
H	0.83	0.18	0.18	0.18	0.18	0.18	0.18							
I	0.83	0.90	0.90	0.90	0.90	0.91	0.90	1.40						
J	0.82	0.16	0.16	0.16	0.16	0.16	0.16	0.16	1.42					
K	0.82	0.16	0.10	0.16	0.16	0.16	0.16	0.16	1.41	0.001				
L	0.75	0.00	0.00	0.00	0.00	0.003	0.00	0.18	0.90	0.16	0.16			
M	0.00	0.75	0.75	0.75	0.75	0.75	0.75	1.46	1.50	1.55	1.50	0.75		
N	0.74	0.01	0.01	0.01	0.01	0.01	0.01	0.18	0.91	0.15	0.15	0.01	0.74	

A = PZ409970.1, B = PZ411705.1, C = PZ413041.1, D = PZ413044.1, E = PZ413043.1, F = PZ469357.1, G = PZ413047.1, H = NC010526.1, I = OZ284400.1, J = MG518346.1, K = MG518348.1, L = MZ674495.1, M = NC024678.1, N = KX171197.1

genetics and phylogenetic studies. Similar deposition of *COI* sequences in GenBank was reported by Ros and Breeuwer (2007).

Determination of Nucleotide Composition

The nucleotide composition analysis of the *COI* I and *COI* II sequences revealed a high A+T content in all *Tetranychus* isolates. The A+T content ranged from 72.50 to 75.37 per cent for *COI* I and 74.53 to 77.56 per cent for *COI* II, whereas the G+C content remained comparatively low (Table 3). The predominance of A+T nucleotides is a characteristic feature of arthropod mitochondrial genomes and agrees with earlier reports on *Tetranychus* species (Navajas *et al.*, 1996a; Ros and Breeuwer 2007).

Barcode Gap Analysis

The *COI* I and *COI* II gene sequences of spider mite collected from different agro-climatic zones of Gujarat were aligned using the ClustalW algorithm implemented in BioEdit v7. The alignment revealed a high degree of sequence conservation across all populations, with only a few nucleotide substitutions observed in accession numbers PZ412362.1 (Narmada) and PZ409970.1 (Navsari) at specific positions (Fig. 3). Most nucleotide sites remained conserved among individuals, indicating limited genetic variation within the species. The observed single nucleotide polymorphisms (SNPs) represent minor intraspecific divergence and suggest substantial genetic homogeneity among spider mite populations from different geographical regions of Gujarat. Similar findings were reported by (Bennur, 2015) low intraspecific barcode divergence in spider mites.

Phylogenetic Analysis and Genetic Distance

Maximum Likelihood phylogenetic analysis based on *COI* I and *COI* II sequences grouped the Gujarat isolates with reference *Tetranychus* sequences retrieved from GenBank. Two major clades were observed, with most Gujarat isolates clustering together, while the Narmada isolate formed a distinct branch in *COI* I and Navsari *COI* II because of its relatively higher sequence divergence (Fig. 4). The clustering pattern confirmed the close genetic relationship among the collected isolates and supported their taxonomic identification. Similar phylogenetic relationships were reported by (Navajas *et al.*, 1996b; Ros and Breeuwer, 2007). Pairwise genetic distance analysis of the *COI* I and *COI* II sequences revealed low genetic divergence among the Gujarat isolates. The genetic distance ranged from 0.00 to 1.46 for *COI* I and 0.00 to 1.55 for *COI* II (Table 4 and Table 5). Most isolates exhibited very low intraspecific divergence, whereas the Narmada isolate showed comparatively higher divergence than the remaining Gujarat isolates. Similar findings were reported by Arunima (2017) and Kadu (2019) who observed low genetic divergence among spider mite populations using mitochondrial *COI* sequences indicating high genetic similarity within species and supporting the usefulness of mitochondrial *COI* markers for DNA barcoding and genetic diversity studies.

Genetic Distance Heatmap Visualization

Heatmaps based on the pairwise genetic distance matrices of the *COI* I and *COI* II sequences were generated to visualize the genetic relationships among the *Tetranychus* isolates (Fig. 5). In both heatmaps, most

Table 6: List of accession numbers and process ID generated for the sequences deposited in NCBI and BOLD respectively

Sr. no.	Sample ID	Gene	Accession No. (NCBI)	Process ID(BOLD)
1	MNCIS1	<i>COI I</i>	PZ406922.1	SDP1222020-26
2	MACIS2	<i>COI I</i>	PZ410424.1	SDP1222021-26
3	MSCIS3	<i>COI I</i>	PZ411843.1	SDP1222022-26
4	MTCIS4	<i>COI I</i>	PZ413040.1	SDP1222046-26
5	MVCIS5	<i>COI I</i>	PZ413042.1	SDP1222025-26
6	MNACIS6	<i>COI I</i>	PZ412362.1	SDP1222047-26
7	MBCIS7	<i>COI I</i>	PZ413045.1	SDP1222026-26
8	MNCIIS1	<i>COI II</i>	PZ409970.1	SDP1222039-26
9	MACIIS2	<i>COI II</i>	PZ411705.1	SDP1222040-26
10	MSCIIS3	<i>COI II</i>	PZ413041.1	SDP1222041-26
11	MTCIIS4	<i>COI II</i>	PZ413044.1	SDP1222042-26
12	MVCIIS5	<i>COI II</i>	PZ413043.1	SDP1222043-26
13	MNACIIS6	<i>COI II</i>	PZ469357.1	SDP1222044-26
14	MBCIIS7	<i>COI II</i>	PZ413047.1	SDP1222045-26

Gujarat isolates exhibited low genetic divergence indicating a high degree of genetic similarity. However, the Narmada (PZ412362.1) isolate showed relatively higher divergence than the remaining isolates, which was consistent with the pairwise genetic distance and phylogenetic analyses. The clustering pattern observed in both *COI I* and *COI II* heatmaps further confirmed the close genetic relationship among the studied isolates and demonstrated the suitability of mitochondrial *COI* markers for DNA barcoding and genetic diversity analysis. Similar patterns of heatmap representation were reported by (Pathak, 2025).

DNA Barcode Submission to BOLD

Specimen data of spider mites collected from different geographical locations of Gujarat including taxonomic information, specimen images, DNA sequence data and trace files were submitted to the Barcode of Life Data

System (BOLD). Unique process IDs were assigned, and *COI I* and *COI II* barcodes generated for reliable identification and comparative analysis (Table 6). As of 18 June 2026, a total 674 published barcode records of *T. urticae*, 6 records of *T. malaysiensis* and 1 published record of *T. macfarlanei* were available in the database, to which the present study contributed two additional barcode sequences for *T. urticae*, six barcode sequences for *T. malaysiensis* and six barcode sequences for *T. macfarlanei*. The records generated in the present study constitute the first DNA barcode records of spider mites from Gujarat, India to be published in the Barcode of Life Data System (BOLD). Similar barcode data submissions were reported by Kadu (2019), Arunima (2017) for spider mites and Pathak (2025) for *Spodoptera litura*.

Conclusion

The present study successfully employed mitochondrial *COI I* and *COI II* gene markers for the molecular characterization and DNA barcoding of spider mite associated with okra from different agro-climatic regions of Gujarat. High-quality DNA and successful PCR amplification enabled reliable sequencing and molecular identification of all isolates. BLAST analysis confirmed the identity of the collected spider mites, while phylogenetic analysis, pairwise genetic distance and heatmap analyses revealed a close genetic relationship among most Gujarat isolates, with relatively higher divergence observed in the Narmada isolate. The high A+T content observed in both *COI* markers was consistent with the typical mitochondrial genome composition of tetranychid mites. The generated sequences were successfully submitted to GenBank and the Barcode of Life Data Systems (BOLD) providing valuable DNA barcode records for future taxonomic, phylogenetic and biodiversity studies. Overall, the study demonstrated that mitochondrial *COI* markers are effective tools for accurate identification and genetic characterization of spider mite and provide a strong molecular foundation for future research and management of spider mites in okra.

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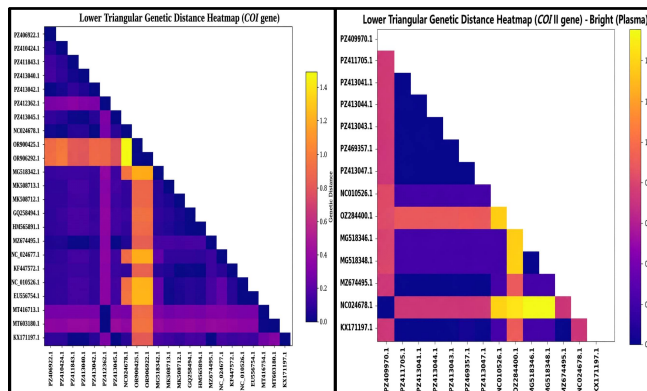


Fig. 5: Heatmap of pairwise genetic distances among spider mite samples based on partial mitochondrial cytochrome oxidase (*COI I* and *COI II*) gene sequences.

and guidance throughout the course of this experimental program.

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