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FIRST MOLECULAR CONFIRMATION OF *Liriomyza sativae* (BLANCHARD) (DIPTERA: AGROMYZIDAE) INFESTING TOMATO (*Solanum lycopersicum* L.) IN GUJARAT, INDIA

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ABSTRACT

Leaf miner is an economically important invasive pest causing considerable damage to tomato. The present investigation aimed to confirm the identity of leaf miner infesting tomato (*Solanum lycopersicum* L.) in Anand, Gujarat, India, through DNA barcoding using the mitochondrial cytochrome c oxidase subunit I (*COI*) gene. Genomic DNA extracted from adult specimens was amplified using universal Folmer primers and the PCR products (688 bp) were sequenced using an ABI-3130 capillary DNA sequencer. The obtained sequence was submitted to the NCBI GenBank database (Accession No. PZ245525). BLAST analysis showed 99.84% sequence similarity with *L. sativae* isolate Ls42 (MN525176.1), while phylogenetic analysis clustered the present isolate with authenticated *L. sativae* sequences, confirming its taxonomic identity. This study provides the first confirmed molecular report of *L. sativae* infesting tomato in Anand, Gujarat, India and establishes the utility of the mitochondrial *COI* gene as a reliable marker for species identification.

Key words : Leaf miner, *Liriomyza sativae*, Molecular characterization, DNA barcoding, *COI* gene, NCBI-BLAST, Phylogenetic analysis, Tomato.

Introduction

Tomato is one of the most important and economically remunerative vegetable crops cultivated worldwide. It is regarded as a protective food owing to its high nutritional value, being a rich source of vitamins (A and C), minerals, antioxidants and lycopene and is extensively grown across diverse agro-climatic regions (Jain *et al.*, 2013).

Within India, tomato is cultivated extensively across diverse agro-climatic regions under both irrigated and rainfed conditions. According to the latest horticultural statistics of the Government of India, Madhya Pradesh is the leading tomato-producing state, followed by Andhra Pradesh, Karnataka, Tamil Nadu, Odisha and Gujarat, which together contribute a major share of the country's total tomato production (Anonymous, 2025). Gujarat is one of the important tomato-producing states of India, where the crop is cultivated widely in districts such as

Anand, Kheda, Gandhinagar, Banaskantha, Sabarkantha, Dahod, Narmada, Vadodara, Valsad, Bhavnagar and Dang (Vala *et al.*, 2021).

Tomato plants are attacked by number of insect pests throughout their growth stages. In which several insect species have been identified as key pests of tomato worldwide, including whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), which acts as a vector of Tomato Yellow Leaf Curl Virus (TYLCV); jassid, *Empoasca fabae* (Harris) (Homoptera: Cicadellidae); South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae); fruit borer, *Helicoverpa armigera* (Hubner) Hardwick (Lepidoptera : Noctuidae); leaf miner, *Liriomyza sativae* (Blanchard) (Diptera: Agromyzidae) serpentine leaf miner, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae); onion thrips, *Thrips tabaci* (Lindeman) (Thysanoptera: Thripidae); and

cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) (Picanco *et al.*, 2007).

Among the insect pests infesting tomato, leaf miners belonging to the genus *Liriomyza* (Diptera: Agromyzidae) have gained considerable economic importance due to their wide host range and destructive feeding habit. Species of *Liriomyza* are highly polyphagous and infest a large number of vegetable, ornamental and field crops worldwide (Parrella, 1987; Spencer, 1973). Srinivasan *et al.* (1995) reported that leaf miner was distributed widely in South India and infested 78 host plant species belonging to various agricultural, horticultural and ornamental crops, with tomato, cotton, pulses, chrysanthemum and cucurbits being among the major hosts. Subsequent advances in taxonomic and molecular identification techniques revealed that more than one species of *Liriomyza* occurs on vegetable crops in India. As noted by Spencer (1990), the close morphological similarity among species often leads to misidentification based solely on external characters. Consequently, many earlier records attributed to *L. trifolii* were later found to involve *L. sativae*. Molecular studies carried out by Firake *et al.* (2018) confirmed the occurrence and widespread distribution of *L. sativae* on vegetable crops in India, establishing it as one of the predominant leaf miner species associated with tomato cultivation.

Materials and Methods

Molecular identification is the latest technique aiming at the identification of new specimens by assessing their DNA sequence similarity. The amplification of mitochondrial cytochrome oxidase I (*COI*) gene fragments using the universal primers of Folmer *et al.* (1994) is generally considered a reliable, cost-effective and easy molecular identification tool with wide applicability across metazoan taxa.

Specimens of leaf miner were collected and brought to the Biological Control Research Laboratory, ICAR Unit-9, Anand Agricultural University, Anand during 2026 for further study. Identification of this pest was confirmed through molecular analysis, marking this may be as first time in Gujarat. Molecular characterization study was conducted SLS Research Pvt. Ltd., Surat, Gujarat. Adult were used for DNA extraction. Genomic DNA was isolated by using DNA extraction kit. PCR amplification of *COI* (cytochrome oxidase subunit I) gene was performed by following the standard protocol Folmer *et al.* (1994). The amplified products were sequenced by capillary sequencer ABI-3130. Gene sequence was checked for homology by using NCBI-BLAST.

Results and Discussion

Mitochondrial *COXI* gene sequences of *L. sativae*

The GenBank accession number PZ245525 corresponds to the partial coding sequence of the mitochondrial cytochrome c oxidase subunit I (*COXI*) gene from *Liriomyza sativae* isolate BACA-1

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GGCCAAAAAATCAAAATAAATGTTGGTATAA
AATTGGGTCTCCTCCACCGGCTGGATCAAAA
AATGATGTATTAAAATTTTCGATCTGTTAATAAT
ATAGTAATTGCTCCAGCTAGCACAGGCAATGA
TAAAAGTAATAATACAGCAGTAATTAATACTGATCACA
CAAATAAAGGTATTCGATCAAACTAATTCCTGTTGATCG
TATATTAATAAATTGTTGTAATAAAATTTACTGTC
CCCTAAAATAGAAAGAAATTTCCAGCTAAAT
GGAGAGAAAAAATAGCTAAATCTACTGAAGCACCAC
CATGTGCAATAATTGAAGAAAGTGGAGGGTAAACC
GTTTCATCCTGTCCCAGCCCCATTTTCTACTATACTGC
TTATTAATAAAGAGTTAAAGCAGGGG
GTAATAATCAAAAACCTATATTATTTATTCGAGGAAATGCT
ATGTCTGGAGCTCCTAATATTAAAGGAACT
AATCAATTACCAATCCTCCAATTATAATAGGTATAACT
ATAAAAAAATTATAATAAAAGCATGAGCAGT
TACAATAACATTATAAATTTGGTCAATACCAATTAAAGCACC
CGGATGTCCTAATTCTGCTCGAATAAGAATTCTAAGA
GAAGTTCCTACTATTCCAGCTCAGGCTCCAAATATAA
TATATAATGTTCCAATATCTTTATGATT.
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Sequence submission and NCBI-BLAST analysis

When the consensus sequence of the mitochondrial cytochrome c oxidase subunit I (*COXI*) gene of *L. sativae* isolate BACA-1 was analyzed for sequence homology using the NCBI Basic Local Alignment Search Tool (BLAST), it exhibited a high degree of similarity with previously reported *COXI* gene sequences of *L. sativae* available in the NCBI GenBank database (Fig. 1). Species authentication through NCBI-BLAST confirmed the identity of the specimen as *L. sativae*. The highest sequence similarity (99.84%) was observed with *L. sativae* isolate Ls42 (GenBank accession number MN525176.1), followed by *L. sativae* isolate Ls47 (MN525177.1) showing 99.68 per cent similarity. Other *L. sativae* sequences available in the GenBank database also exhibited high levels of similarity ranging from 99.35 to 99.84 per cent.

The available *COXI* gene sequences of *L. sativae* were retrieved from the NCBI GenBank database for comparative analysis with the sequence generated in the present study. A total of ten closely related *L. sativae* *COXI* gene sequences, including MN525176.1, MN525177.1, MN525175.1, NC_015926.1, JQ862475.1,

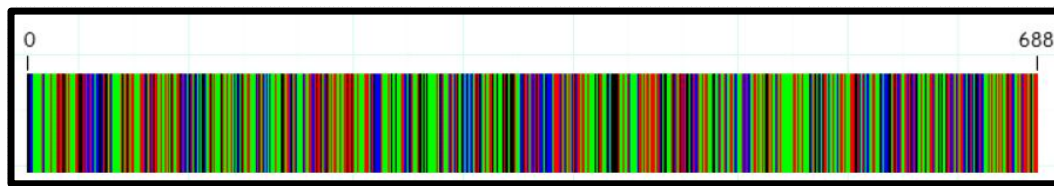


Fig. 1 : DNA barcode image of cytochrome c oxidase subunit I (*COXI*) gene of *L. sativae* BACA-1.

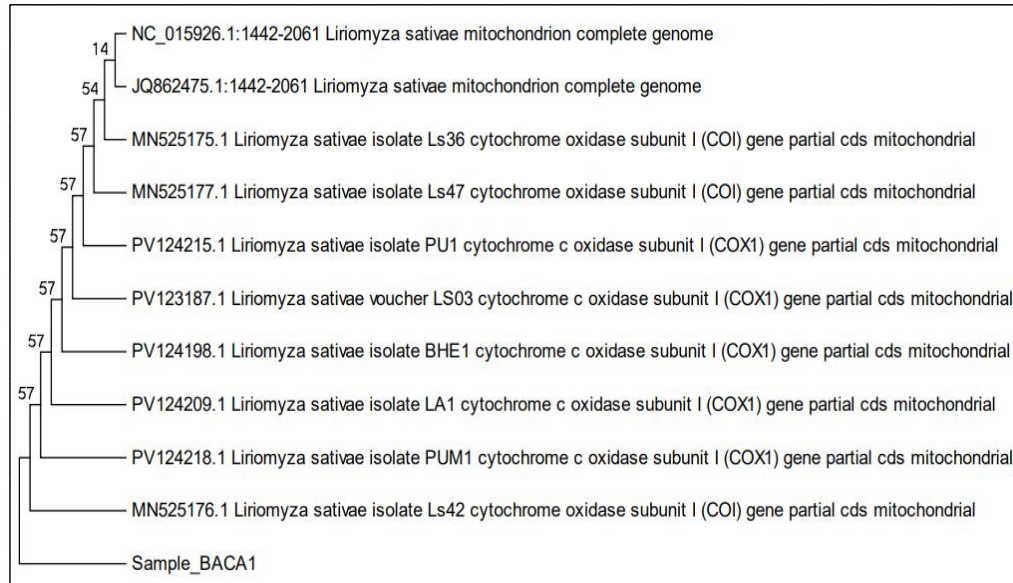


Fig. 2 : Phylogenetic tree constructed for *L. sativae* infesting tomato.

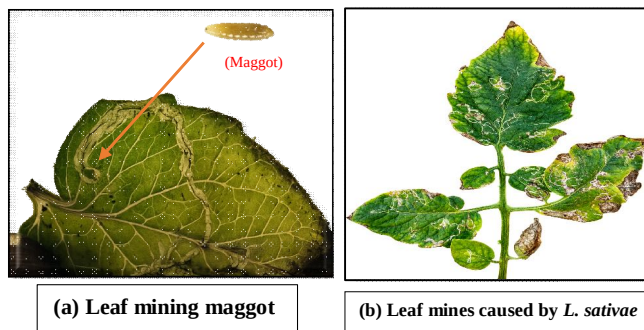


Fig. 3 : Stages of infestation by *L. sativae* on leaves of tomato.

PV124215.1, PV123187.1, PV124198.1, PV124209.1 and PV124218.1 were selected for phylogenetic analysis. An evolutionary tree was constructed using the Neighbor-Joining method (Fig. 2) to determine the relationship between the present isolate and reference sequences retrieved from GenBank. The details of the sequences used for phylogenetic analysis are presented in Table 1.

Phylogenetic analysis

The phylogenetic analysis revealed that *L. sativae* isolate BACA-1 clustered closely with other *L. sativae*

Table 1 : Details of *L. sativae* sequences recorded in India and abroad used for phylogenetic analysis.

S. no.	Description	Accession
1	<i>Liriomyza sativae</i> isolate Ls42 cytochrome oxidase subunit I (<i>COI</i>) gene, partial cds; mitochondrial	MN525176.1
2	<i>Liriomyza sativae</i> isolate Ls47 cytochrome oxidase subunit I (<i>COI</i>) gene, partial cds; mitochondrial	MN525177.1
3	<i>Liriomyza sativae</i> mitochondrion, complete genome	NC_015926.1
4	<i>Liriomyza sativae</i> isolate Ls36 cytochrome oxidase subunit I (<i>COI</i>) gene, partial cds; mitochondrial	MN525175.1
5	<i>Liriomyza sativae</i> isolate PU1 cytochrome c oxidase subunit I (<i>COXI</i>) gene, partial cds; mitochondrial	PV124215.1
6	<i>Liriomyza sativae</i> voucher LS03 cytochrome c oxidase subunit I (<i>COXI</i>) gene, partial cds; mitochondrial	PV123187.1
7	<i>Liriomyza sativae</i> isolate BHE1 cytochrome c oxidase subunit I (<i>COXI</i>) gene, partial cds; mitochondrial	PV124198.1
8	<i>Liriomyza sativae</i> isolate LA1 cytochrome c oxidase subunit I (<i>COXI</i>) gene, partial cds; mitochondrial	PV124209.1
9	<i>Liriomyza sativae</i> isolate PUM1 cytochrome c oxidase subunit I (<i>COXI</i>) gene, partial cds; mitochondrial	PV124218.1
10	<i>Liriomyza sativae</i> mitochondrion, complete genome	JQ862475.1

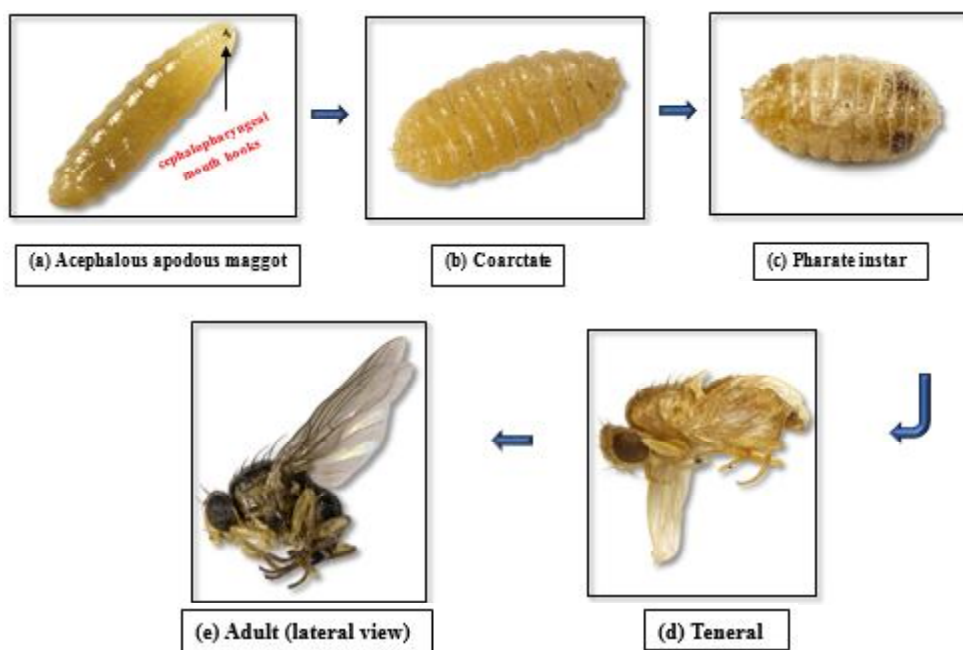


Fig. 4 : Different life stages of *L. sativae* infesting tomato.

isolates and mitochondrial genome sequences available in the NCBI database (Table 1), confirming its taxonomic identity. Sequence alignment indicated a high level of nucleotide conservation among the analyzed isolates, with only minor variations observed across the *COXI* region. The generated *COXI* sequence was deposited in the NCBI GenBank database under accession number PZ245525 and serves as a reliable DNA barcode for molecular identification of *L. sativae*. The findings of the present investigation are in close agreement with those of Srinivasan *et al.* (2026), who employed mitochondrial cytochrome oxidase I (*COXI*) gene sequencing along with species delimitation analyses to characterise *Liriomyza* species and confirmed the pre dominance of *L. sativae* in India. Their phylogenetic analysis grouped *L. sativae* sequences into a distinct cluster, validating the utility of the mt*COI* gene as a reliable molecular marker for species identification and DNA barcoding. Similarly, in the present study, the *COXI* sequence of *L. sativae* isolate BACA-1 showed 99.84 per cent similarity with *L. sativae* isolate Ls42 (MN525176.1) (Tamil Nadu, India) and clustered closely with authenticated *L. sativae* sequences retrieved from the NCBI GenBank database, thereby confirming its taxonomic identity.

Conclusion

The findings of the molecular characterization studies confirmed the species as *Liriomyza sativae* (Blanchard) (Diptera: Agromyzidae). This is the first confirmed molecular report of *Liriomyza sativae* infesting tomato

(*Solanum lycopersicum* L.) in Anand, Gujarat, India.

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