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Genetic diversity of *Tutaabsoluta* (Meyrick, 1917) (Lepidoptera, Gelechiidae) populations in three districts of Tamil Nadu

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Abstract
The present study was carried out to assess the prevalence of *Tutaabsoluta* in vegetable growing regions of Tamil Nadu during September 2016 to August, 2017. Among the twenty four location surveyed in three districts *viz.*, Coimbatore, Dharmapuri and Krishnagiri, *T. absoluta* infestation was found in all locations. Species identification was confirmed by collecting adults from the surveyed locations. Molecular identification of specimens from surveyed districts was performed using mitochondrial (mtDNA) markers. Characterisation of *T. absoluta* populations from Coimbatore, Dharmapuri and Krishnagiri Districts using cytochrome c oxidase subunit I (COI) revealed that the Dharmapuri and Krishnagiri population were closely related with NCBI accession sourced from IARI (KY619683), New Delhi, India, whereas, the Coimbatore population with Spain (GU353336) and Turkey (KTO02421) population.

Keywords: *Tuta absoluta*, survey, tomato, molecular characterisation, COI

Introduction
The South American tomato moth *Tutaabsoluta* (Meyrick, 1917) (Lepidoptera, Gelechiidae) is one of the most devastating invasive pests of tomatoes, both in greenhouse and open field locations in South America, Europe and North Africa (Desneux *et al.*2010) [3]. It has also been reported to attack potatoes (*Solanum tuberosum* L.), eggplants (*Solanum melongena* L.), peppers (*Capsicum annum* L.), weeds (*Datura stramonium* L., *Nicotiana glauca* G.) (Korycinska& Moran 2009) [7] and green beans (*Phaseolus vulgaris* L.) (EPPO 2009) [4]. *T. absoluta* spreads mostly from one tomato plant to another while these are being planted, and from one region to another as a result of the trans-border trade in tomatoes (Tosevski *et al.* 2011 [9], Bettaibi *et al*. 2012) [2]. Incidence of *T. absoluta* was recorded for the first time ontomato at the Indian Institute of Horticultural Research (IIHR), Hessaraghatta, Bengaluru (13°8.12"N 77°29.45"E, altitude 890 m), Karnataka, India during the *Rabi* season of 2014 (Sridhar *et al.*, 2014 [8] Since the initial detection, tomato leaf miner has become the most serious pest causing severe damage on tomato in invaded areas. Conspicuous economic losses and rapid spreading along the areas of traditional tomato production, project this pest as the most seriousagricultural threat to tomato production (Germain, *et al*. 2009) [5]. *T. absoluta* is a serious threat to tomato production, as it can decrease fruit quality and result in 50 to 100% yield losses (Viggniani *et al*. 2009) [10]. The larvae destroy tomato plants by mining the leaves, stems and buds, and by burrowing tunnels in the fruits, thereby rendering fresh tomatoes unmarketable. This pest is of great economic importance and has spread to other regions of Europe. The level of genetic diversity in introduced populations of an invasive species is considered as a major factor influencing its survival and adaptation capacities. Hence, the present study was undertaken to study the genetic diversity of *T. absoluta* populations in Tamil Nadu.

Materials and Methods
Survey conducted at three major tomato growing areas mooted the interest to assess the identity of the insects prevailing in three ecological zones. Hence, insect samples collected from these locations were subjected to molecular characterization (Plate 1).
Genomic DNA was isolated from single adult of *T. absoluta* collectedfrom three different locations following the CTAB method (Sridhar *et al.*, 2014) [8]. The DNA extraction buffer contained 100mM Tris. HCl (pH 8), 10mM EDTA, 1.4M NaCl, 2.0 per cent CTAB and 5.0 per cent β-mercaptoethanol. Individual insect samples were homogenized with 200 µl of DNA extraction buffer taken in test tubes and incubated at 65°C for 1 h in water-bath.

The tubes were removed from the water bath and allowed to cool at room temperature. Equal volume of chloroform and isoamyl alcohol mixture (24:1, v/v) (0.8 volume) were mixed by inversion for 10 min to form an emulsion, centrifuged at 12,000 rpm for 10 min and the clear aqueous phase was transferred to a new sterile tube. Equal volume of ice-cold isopropanol (500 µl) was added and mixed gently by inversion, stored at -20° C for overnight then centrifuged at 12,000 rpm for 10 min to pellet the DNA and the supernatant was discarded. The DNA pellet was washed with 70 per cent % ethanol. After washing DNA pellet was air dried and dissolved in 20-40 µl of Tris-EDTA buffer depending on size of the pellet and stored at -20°C until use.

Quality of genomic DNA was checked by 0.8 per cent agarose gel. Agarose at 0.8gm was dissolved in 100 ml of 1X Tris-Borate-EDTA buffer. After cooling, 1-2 µl ethidium bromide was added from the stock (10 mg ethidium bromide / ml H_2O). Then the mixture was poured into a preset template kept with appropriate comb to make wells. Exactly 2µl of DNA added with 2 µl of loading dye (6X loading dye) were loaded in each well. Electrophoresis was carried out at 65 V for 1 h. Amplified genomic DNA was visualized on UV trans-illuminator (Bio-Rad, USA) and documented using gel documentation system (GELSTAN 1312). The quantification of DNA was done using Nanodrop Spectrophotometer (ND-1000). Based on the nanodrop readings, DNA dilutions were made in TE buffer or double distilled water to make a final concentration of 50-60 ng μl^{-1} and stored at 40° C for further use.

Polymerase chain reaction

The primers mtCOI forward 5'TTGATTTTTTGGTCATCCAGAA 3' and reverse 5' TCCAATGCACTAATCTGCCAT 3' were used to identify the mitochondrial DNA. The PCR was performed in a thermocycler (Eppendorf). A typical reaction mixture had following compositions.

PCR was performed in 25 µl reaction volume containing 12.5 µl of 2 X smART master mix, Cat. No. 280311 (readymade mix of taq polymerase, dNTPs and PCR buffer), 5 µl of template DNA, 3.5 µl of sterile distilled water and 2 µl of each forward and reverse primer.

PCR conditions for amplification were initial denaturation at 94 °C for 3 minutes, followed by 40 cycles each consists of denaturation for 30 seconds at 94 °C, annealing for 40 seconds at 53 °C with final extension for one minute at 72 °C followed by final elongation for 20 minutes at 72 °C.

Sequencing of PCR product

The unpurified amplified PCR product (20 µl) was sent to the sequencing facility, J.K Scientific Company, Coimbatore for single pass DNA sequencing with mtCOI forward and reverse primers (5 µl for each sample).

Sequences analysis

Partial nucleotide sequences of the *T. absoluta* were aligned using MUSCLE (Edgar) and percentage pair wise nucleotide identity plot was made using SDTv 1.0 program (http://web.cbio.uct.ac.za/SDT).

A dendrogram was generated by aligning the selected Partial nucleotide sequences of the *T. absoluta* through Clustal W and tree was prepared using MEGA4 (version 4.0.2). Bootstrap analysis with 1000 replication was done using neighbour joining method which revealed the evolutionary distance between the sequences

Results and Discussion

The DNA of the *T. Absoluta* populations from different locations were amplified using *mitochondrial Cytochrome Oxidase I (mtCOI)* primer. The amplified product length was approximately 670 bp in all the three populations. The dendrogram constructed using *mtCOI* sequences inferred that the three population *viz.*, Coimbatore, Dharmapuri and Krishnagiri were grouped under single cluster and were more closely related (Plate 2). Dharmapuri and Krishnagiri population were closely related with NCBI accession sourced from IARI (KY619683), New Delhi, India, whereas, the Coimbatore population with Spain (GU353336) and Turkey (KTO02421) population. Identification system based on DNA barcoding facilitate the identification of known as well as discovery of new species. DNA barcoding as based on the premise that sequence diversity within a short, standardized segment of the genome which enables identification at the species level (Hebert *et al*., 2003) [6]. In present study, three district populations of *T. absoluta* was characterized using standardized cytochrome c oxidase subunit I (COI) sequencing approach. The three location population sequences were compared with reference sequences available in NCBI GENBANK and analysed by BLAST. Based on the dendrogram, three population were grouped under single cluster and were more closely related. Coimbatore population matches with Spain (GU353336) and Turkey (KTO02421) while Dharmapuri and Krishnagiri population with IARI (KY619683) New Delhi, Similarly, Balagi *et al.* 2015 [1] observed that Coimbatore population with Turkey and Dharmapuri and Krishnagiri with Pune population. Sridhar *et al*. (2014) [8] also analysed thediversity of *T. absoluta* from different locations viz., Shivakote, Madhugirihalli, Thirumalapura, Thamarasanahalli, Agrahara, Gudadhalli, Ivarakandapura (IIHR), Kalenahalli, Linganahalli and Hessaraghatta of Karnataka using mtCOI. According to them, all the *T. absoluta* populations were grouped under single clade in Neighbour-Joining (NJ) tree revealing no genetic variation within populations. Tosevski *et al*. (2011) [9] also analysed the diversity of *T. absoluta* from different villages viz., Donjivrtogos and Navalin villages of South Serbia using mtCOI gene. According to them, COI barcode region of their population had cent per cent identity with the barcode sequence of *T. absoluta* from France (HQ968678; BOLD: PHLAB662-10).

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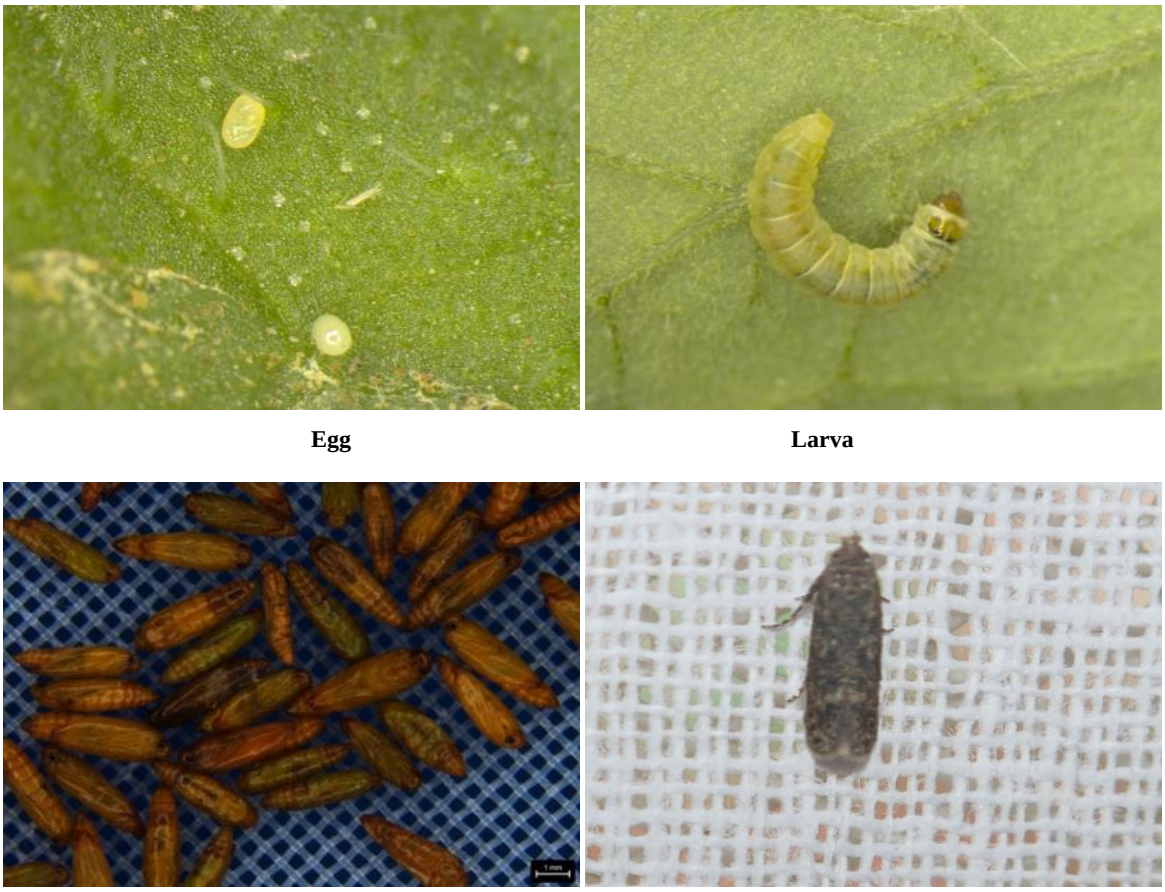

Egg **Larva**

Plate 1: *Tuta absoluta* life stages

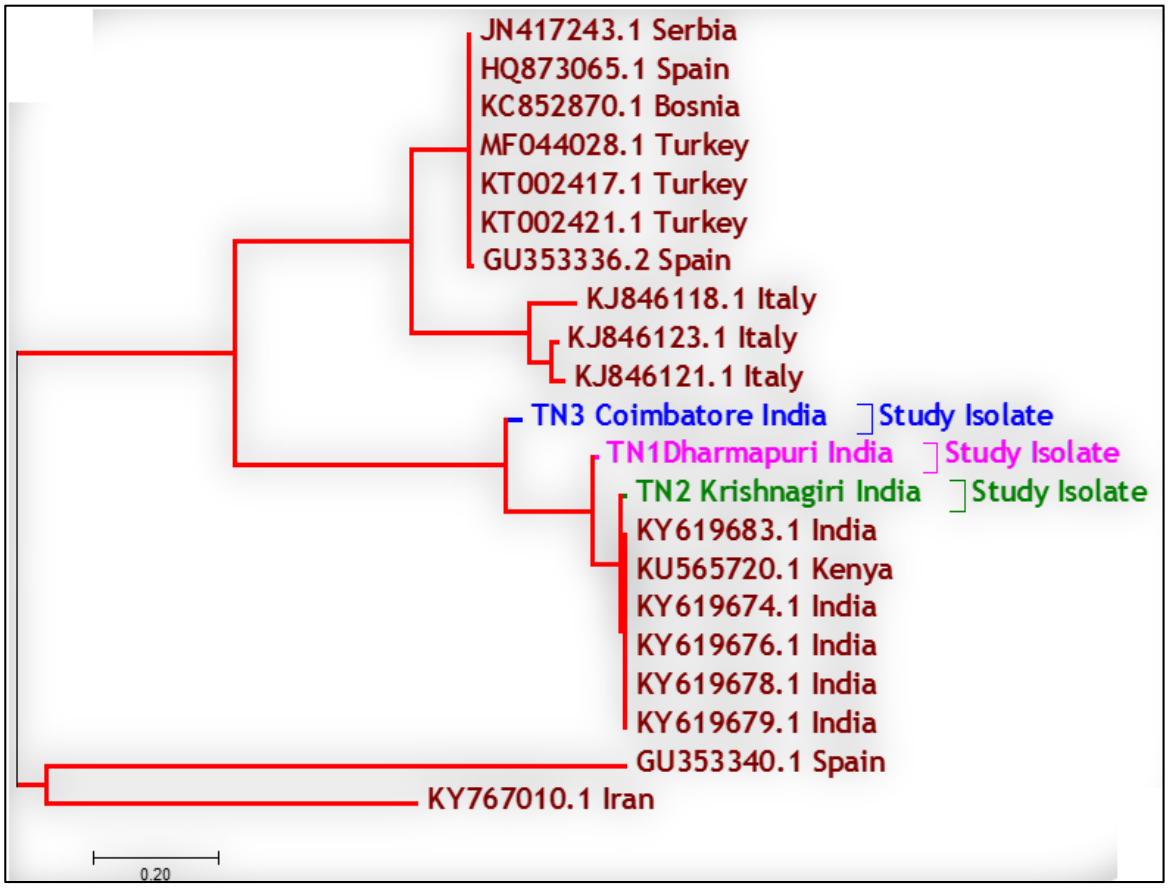

Plate 2: Phylogenetic tree generated from aligned partial mtCOI nucleotide sequences of *Tutaabsoluta.*

Tree was generated by neighbour joining method by aligning the sequences in MEGA 7 using ClustalW. Vertical branches are arbitrary; horizontal branches are proportional to calculated mutation distances; values at nodes indicate percentage boot straps values (1000 replicates).

Coimbatore population

AACTGTCTATCCCCCCCTATCTTCTAATATTGCTCATGGAGGTAGTTCGGATCGAAATTTAAATACATCATTCTTTGA
TCCTGCAGGTGGAGGAGACCCAATTTTATATCAACATTTATTTTGATTTTTTGGGACATCCAGAAGTTTATATTTTAA
GGTAGTTCAGTAGATTTAGCTATTTTTTCTTTACATTTAGCCTGGTATTTCATCGATTTTCTCATGGAGGTAGCCCAG
TAGATTTAGCTTTTTTTCTTTACATTTAGCTGGTATTTCCTCAATTTTAGGAGCTATTAATTTTATTACCACTATTATT
AATATACGAGTTAATGGACTTTCATTTGATCAAATTACCTTTATTTGTTTGAGCCAGTTTTAGCTGGAGCTATCACA
ATATTATTTAACGGACCGAAATTTAAATACATCTTTCTTTGACCCGGCAGGTGG

Dharmapuri population

TTTTGATTCTTACCTCCCTCTCTCACATTACTAATTTCAAACGAATCGTAGAAATGGAGCATGGACTGGTTGACTGT
CTATCCCCCCCTATCTTCTGTTATGCTCATGGAGGTAGCCCAGTAGATTTAGCTTTTTTTCTTTACATTTAGCTGGTA
TTTCCTCAATTTTAGGAGCTATTAATTTTATTACCACTATTATTAATATACGAGTTAATGGACTTTCATTTGATCAAA
TTACCTTTATTTGTTTGAGCTGTTGGTTTACTGCTTTACTCCTTCTTTTATCTTTGCCAGTTTTAGCTGGAGCTATCAC
AATATTATTTAACGGACCGAAATTTAAATACATCTTTCTTTGACCCGGCAGGTGGAGGAAACCCAATTTTATATCAA
CATTTATTTTGATTTTTTGGGACATCCAAAAGTTTATATTTATTCTTTGCCTGA

Krishnagiri population

TTCTTACCCCCCTCTCTCACCCTTTTAATTTCAAGAAGAATCGTAAAAATGGAGCAGGGACTGGTTGAACTGTCTAT
CCCCCCCTATCTTCTAATATTGCTCATGGAGGTAGTTCAGTAGATTTAGCTATTTTTTCTTTACATTTAGCCTGGTAT
TTCATCGATTTTAGGAGCTATTAATTTTATTACCACTATTATTAATATACGAGTTAATGGACTTTCATTTGATCAAAT
ACCCTTTATTTGTTTGAGCTGTTGGTATTACTGCTTTACTCCTTCTTTTATCATTGCCAGTTTTAGCTGGAGCTATCAC
AATATTATTAAACGGATCGAAATTTAAATACATCATTCTTTGATCCTGCAGGTGGAGGAGACCCAATTTTATATCAA
CATTTATTTTGATTTTTTGGGACATCCAGAAGTTTATATTTTAATCCTTGCCTGA

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