Genetic homogeneity in South American tomato pinworm, *Tuta absoluta*: a new invasive pest to oriental region

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Abstract

South American tomato leaf miner, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), is an important invasive pest of Tomato which invaded India and Nepal in 2014 and 2016, respectively. In the present study, samples from five localities of India and one from Nepal were used for the investigation of genetic diversity of *T. absoluta* by employing a fragment in the mtDNA gene-encoding cytochrome oxidase I (COI). Based on the partial COI gene, high genetic homogeneity was detected in *T. absoluta* populations of India and Nepal with rest of the world. Less nucleotide diversity (π 0.00137) was also detected in the populations of *T. absoluta* from different countries. This is first attempt to analyze molecular data for this new invasive species from India and Nepal.

Keywords Cytochrome oxidase · Invasive pest · India · Nepal · Tomato · *Tuta absoluta*

Introduction

Tomato (*Lycopersicon esculentum* Miller) is one of the most important vegetable crops in the world and particularly in India. In India, its production has increased 36 times from a mere 0.54 Mt in 1961 to 18.7 Mt in 2015–2016. At present, India is the second largest producer, accounting for 11.2% of the world production and the second largest in terms of acreage accounting for 18.27% (IHD 2017).

South American tomato pinworm, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), also known as the tomato leaf miner is an oligophagous pest associated with solanaceous crops. It causes reductions in yield and fruit quality to the tune of 50–100% loss in either greenhouses

or fields. Plants are damaged by direct feeding on leaves, stems, buds, calyx, young fruits, and also on ripe fruits. Furthermore, damage is also caused by the invasion of secondary pathogens which enter through the wounds made by the pest (EPPO 2005). There are several host plants infested by *T. absoluta*, the primary host is tomato, but damage has been reported on some other secondary hosts like eggplant, *Solanum melongena* L.; pepper, *Capsicum annuum* L.; potato, *Solanum tuberosum* L.; sweet pepper, *S. muricatum* L.; tobacco, *Nicotiana tabacum* L. (Vargas 1970; Pereyra and Sánchez 2006). This moth has also been reported from several solanaceous weeds, viz., *Datura ferox* L., *D. stramonium* L. and *N. glauca* Graham (Larrain 1986; Desneux et al. 2010).

T. absoluta was accidentally introduced to Spain in 2006, from where it spread North to The Netherlands and East to Iran (Desneux et al. 2010, 2011; Baniamერი and Cheraghian 2012). Following its introduction up to Afro-Eurasia in 2010, this pest was in the quarantine watch list of India. In October, 2014, this pest was first detected infesting tomato fields in Pune, Ahmednagar, Dhule, Jalgaon, Nashik, and Satara Districts of Maharashtra, India (Shashank et al. 2015). Subsequently, pest was recorded from Karnataka (ICAR 2015; Kalleshwaraswamy et al. 2015; Ballal et al. 2016), Tamil Nadu (Shanmugam et al. 2016; Ballal et al. 2016), Andhra Pradesh, Telangana (Kumari et al. 2015), Gujarat (Ballal et al. 2016; Chavan et al. 2016; Per. Comm.),

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North India (Shashank et al. 2016), and Northeastern India (Sankarganesh et al. 2017). Since its introduction to India, the possibility of invasion to Nepal was always existed due to open border, weak quarantine, and import of tomato from India. In May 2016, this pest was first reported from Nepal (Bajracharya et al. 2016). The report of these two invasions from India and Nepal is the first report from oriental region.

The success of an invasive species such as *T. absoluta* may be determined by both biotic and abiotic factors that promote its adaptation and spread through a wide geographical area (Cifuentes et al. 2011). Assessing the genetic structure and diversity of a invasive species population with native ones using molecular markers helps in understanding gene flow, migration rates, isolation among populations, and other micro-evolutionary processes related with genetic differentiation (Ito et al. 2011; Hufbauer and Roderick 2005). Many studies have shown that the population genetic structure and molecular diversity of most species would change in new habitats, it is observed obviously in some invasive species (Hoos et al. 2010; Rubinoff et al. 2011). Because, mitochondrial DNA is variable and has strict maternal heredity and no genetic recombination, it is ideal for examining population genetic structure and tracing the history of variations in organism (Trewick 2000; Gissi et al. 2008; Shashank et al. 2014; Sarma et al. 2016). In addition, mtDNA provides a good indication of intra- and inter-population variations (Vogler et al. 1993; Margam et al. 2011).

As a successive invasive species, *T. absoluta* has invaded major tomato growing areas of India (Shashank et al. 2015, 2016) and Nepal (Bajracharya et al. 2016). However, nothing is known about the existence of the wild population of *T. absoluta* in India. In view of its potential for economic damage and spread, the monitoring of the invasion of *T. absoluta* is critical and to know the point/source of entry of a population in to new country is very important. The aim of this study is to describe the basic information for molecular variability and population genetic structure of *T. absoluta* using mitochondrial marker cytochrome C oxidase (COI).

Methods

The larvae of the *T. absoluta* were collected from five localities (Pusa campus, Delhi; Pune, Maharashtra; Hyderabad, Telangana; Bangalore, Karnataka; Tapi, Gujarat) in India and one locality in Nepal (Balaju, Kathmandu) (details in Table 1). The larvae were morphologically identified as *T. absoluta* using diagnostic characters provided by Hayden et al. (2013).

Genomic DNA was isolated from larvae using a DNeasy animal tissue kit following the manufacturer's instructions (Qiagen, Valencia, CA). The rest of the larvae were preserved as the specimen voucher in the National Pusa

Collection (NPC), Division of Entomology, ICAR-Indian Agricultural Research Institute (IARI), New Delhi. The genomic DNA was visualized using 0.8% agarose gel and quantified by fluorometer using the standard procedures. A portion of the total DNA was preserved in -70°C for future reference purposes. The universal barcode primer described by Folmer et al. (1994) (LCO-50-GGT CAA CAA ATC ATA AAG ATA TTG G-30; HCO-50-TAA ACT TCA GGG TGA CCA AAA AAT CA-30) specific to mitochondrial cytochrome oxidase I (COI) was used in the present study. The optimized PCR conditions (per 24 μL) using 0.1 μL Dream Taq DNA polymerase (5U/ μL) (Thermo Scientific, Lithuania) were 2.5 μL of 10X PCR buffer with 2 μL of 25 mM MgCl_2 , 0.5 μL of 10 mM dNTPs (Thermo Scientific, Lithuania), 0.5 μL each of forward and reverse primers, 16 μL of nuclease-free water (Ambion, USA). The PCR (Applied Biosystems® Veriti® 96-Well Thermal Cycler) conditions were as follows: the initial denaturation for 4 min at 94°C followed by 35 cycles of denaturing for 30 s at 94°C , annealing for 1 min at 47°C , and extension time of 50 s at 72°C , with a final extension for 8 min at 72°C . The PCR products were visualized using AlphaImager® HP (AlphaView Version 3.2.2.0) on agarose gel after electrophoresis (SCIE-PLAS HU10 Mini-Plus horizontal). Purified PCR products are sequenced in an automated sequencer (ABI Prism 3730; Applied Biosystems, USA) at the specific commercial facilities (AgriGenome, Hyderabad, India).

Data analysis

All the sequences in FASTA format were imported into the sequence alignment application of MEGA 7.0 (Kumar et al. 2016) software package and multiple sequence alignments were performed with the ClustalW (Jeanmougin et al. 1998) algorithm using the default parameters. The BLAST program was used to search for homologous *T. absoluta* sequences in the NCBI databases (<http://www.ncbi.nlm.nih.gov/>). The sequence details were analyzed carefully and submitted to NCBI for GenBank Accessions (Table 1).

A total of 40 sequences (20 from this study and 20 sequences from different localities were downloaded from NCBI database) were used in diversity analysis. DnaSP 5.10.01 (Librado and Rozas 2009) was used to estimate a series of population-level parameters including pairwise nucleotide diversity π and haplotype diversity h . Neutrality tests like Tajima's D (1989), Fu and Li's D (1993), and Fu's FS (1997), were also calculated by DnaSP 5.10.01 (Librado and Rozas 2009) for investigating the historical population demographics and testing whether the sequences conformed to the expectations of neutrality. We calculated pairwise F_{ST} using Arlequin 3.5 (Excoffier and Lischer 2010), which accurately reflects the patterns of genetic variation.

Table 1 Details of the sample data used in the analyses of *T. absoluta* population

Continents and countries	Accession no.	Code	Specific location	Latitude	Longitude
Oriental					
India					
Delhi	KY619685	DEL1	Indian Agricultural Research Institute, New Delhi	28.6282	77.1602
	KY619686	DEL2			
	KY619687	DEL3			
Maharashtra	KP814055	MHA1	IARI-Regional Station, Pune		
	KP814056	MHA2			
	KP814057	MHA3			
Telangana	KY619681	TEL1	Hyderabad	17.3351	78.4204
	KY619682	TEL 2			
	KY619683	TEL 3			
	KY619684	TEL 4			
Karnataka	KP793741	BAN1	IIHR, Bangalore		
	KP793742	BAN2			
Gujarat	KY619673	GUJ1	Tapi, Gujarat	21.0954	73.3916
	KY619674	GUJ2			
	KY619675	GUJ3			
	KY619676	GUJ4			
Nepal	KY619677	NEP1	Balaju, Kathmandu	27.7313	85.2945
	KY619678	NEP2			
	KY619679	NEP3			
	KY619680	NEP4			
Europe					
Serbia	JN417242	SER1	Leskovac, Serbia	—	—
France	HQ968678	FRA1	Provence-Alpes-Cote d’Azur, France	—	—
Bosnia and Herzegovina	KC852871	BOH1	Bijeljina, Bosnia, and Herzegovina	—	—
Montenegro	KC852872	MON2	Ulcinj, Montenegro	—	—
South America					
Peru	KX443108	PER1	International Potato Center (CIP), Lima, Peru	—	—
	KX443110	PER2			
	KX443111	PER3			
Africa					
Tunisia	JQ749676	TUN1		—	—
	JQ749677	TUN2			
	JQ749678	TUN3			
	JQ749679	TUN4			
	JQ749680	TUN5			
	JQ749681	TUN6			
	JQ749682	TUN7			
	JQ749683	TUN8			
	JQ749684	TUN9			
	JQ749685	TUN10			
Kenya	KP324752	KEN1	Isiolo, Kenya	—	—
	KP324753	KEN2	Isiolo, Kenya	—	—
Middle east				—	—
Oman	KT452897.1	OMA1		—	—
Out groups					
<i>Bombyx mori</i>	KU205352.1				
<i>Scrobipalpula sacculicola</i>	KT139410.1				
<i>Scrobipalpula tussilaginis</i>	KX049635.1				
<i>Gnorimoschema herbichii</i>	KT782393.1				
<i>Gnorimoschema subterranea</i>	KT131942.1				

The sequencing data generated in this study is from samples collected from different parts in India and Nepal. Gene was sequenced from 3'–5' direction

Sampling locations were grouped according to historical records of *T. absoluta* distribution and invasion, resulting in the following combinations: rest of the world (Table 1) and Oriental (India–Nepal).

Results

Genetic variability of COI sequences

Twenty individuals of *T. absoluta* breeding on tomato were collected from five different localities of India and one from Nepal. Other 20 sequences were downloaded from NCBI database from eight countries based on the spread of *T. absoluta* (Table 1). The sample size disparity is mainly based on lack of sequence information from different countries. Totally, 40 sequences were used for analysis. The mean total nucleotide composition is A 28.2%, T 39.5%, C 16.5%, and G 15.8% in the sequences. The average AT content is 67.7% and the CG is 32.3%. This high AT content (67.7%) is a general feature of the COI region in arthropods, and is comparable with other studies on insect and mite taxa. Furthermore, test statistics were generated for the 40 COI sequences in which rest of the world group and oriental (India–Nepal) were analyzed as discrete units in the case of the diversity and selective neutrality tests (Table 2). Surprisingly, there is a lack of variation in COI sequences. A total of three haplotypes in rest

of the world group and single haplotype in oriental group were observed. There is a negligible nucleotide diversity (π) 0.00137 among rest of world group which also contains native range, while locations newly invaded that is Oriental group are completely monotypic (Table 2). Demographic history changes were analyzed for two groups through neutrality tests. The AMOVA analysis revealed that 95.19% of total genetic variation was partitioned within populations. The remaining 4.81% was partitioned among populations ($F_{st}=0.04812$): this amount of variance is highly insignificant ($P<0.001$) and reveals that there is no considerable population genetic structure (Table 3).

Phylogenetic analysis

The neighbor-joining tree (NJ tree based on Kimura three-parameter distance at 1000 iterations) was constructed based on 40 sequences including 18 sequences from the present study and 22 sequences from GenBank and outliers (Table 1) using MEGA 7.0. Based on strict consensus NJ tree, all populations cluster together in a single group. The phylogenetic tree had a total branch length of 0.3455 base substitutions per site. The tree shows that there is no clustering associated with geographic origin. However, the samples from the same localities are grouped together with Kenyan samples as a base. This suggests that there is a homogeneity in the *T. absoluta* populations.

Table 2 Genetic variability of COI sequences by group

	Rest of the world	Oriental	Total
Sample size	20	20	40
No. of haplotypes (Nh)	3	1	2
Haplotype diversity (<i>h</i>)	0.195	0	0.099
Nucleotide diversity (π)	0.00137	0	0.00069
No. of segregating sites (<i>S</i>)	7	0	7
Fu and Li's <i>F</i>	−2.68009	N/A	−3.35235 (** $P<0.02$)
Fu and Li's <i>D</i>	−2.45506	N/A	−3.20829 (** $P<0.02$)
Tajima's <i>D</i>	−1.97235	N/A	−2.10017 (* $P<0.05$)
Fu's <i>F</i>	0.695	N/A	−0.212

Rest of the world grouping includes all the sequences from Table 1 except India–Nepal region. For Fu and Li's *F* and *D* and Tajima's *D* values, significant ($P<0.05$) values, while others have $P>0.10$

Table 3 Analysis of molecular variance (AMOVA) for the COI sequences

Source of variation	<i>df</i>	Sum of squares	Variance components	Percentage of variation
Among populations	1	5.025	0.12632 Va	4.81
Within populations	38	94.950	2.49868 Vb	95.19
Total	39	99.975	2.62500	
Fixation Index	F_{ST} : 0.04812			

Discussion

Mitochondrial DNA has been used for elucidating genetic variation, speciation of complex insect species, identification, and origin invasive species (Kruse and Sperling 2001; Armstrong and Ball 2005; Valade et al. 2009; Hafiz and Samreen 2016). Information on genetic diversity and population genetics of invasive species is essential in the context of developing invasive species management strategies (Lee 2002). *Tuta absoluta* is one of the most devastating pest-threatening tomato crops worldwide. In the last decade, this pest has spread and expanded to most of Europe, Africa, and now in Asia, causing extensive damage to the crop itself and to the international tomato trade (Campos et al. 2017). *T. absoluta* introduced to India and Nepal in 2014 and 2015, respectively (Shashank et al. 2015; Bajracharya et al. 2016). However, information on genetic diversity of this invasive species is totally lacking in both the countries. This is the first report of genetic diversity of this pest from India and Nepal using mitochondrial cytochrome oxidase I gene.

The present results recorded high genetic homogeneity among different populations which is evident by less nucleotide variation. Furthermore, the lack of genetic variability was also represented by neighbor-joining tree (Fig. 1). The genetic homogeneity observed in the present results is similar to those of Flores et al. (2003) who also observed high genetic homogeneity in Argentinian populations of *T. absoluta* using allozymic polymorphisms. They concluded that lack of particular alleles or allelic frequencies in populations of *T. absoluta* from arid regions or from subtropical areas hinder characterisation of each population according to its geographic origin. Similar study was conducted by Cifuentes et al. (2011) in *T. absoluta* using cytochrome oxidase I (COI) and internal transcribed spacers 1 (ITS1 and ITS2) to understand genetic diversity of 23 Mediterranean and ten native South American populations. They also observed high genetic homogeneity in *T. absoluta* populations from the Mediterranean Basin and South America, and concluded that a single genetic type was identified in this pest. Genetic homogeneity in recently introduced species in a new habitat is not atypical, similar results have been observed in other arthropods such as *Oryctes rhinoceros* (Reil et al. 2016), *Ochlerotatus caspius* (Porretta et al. 2007), *Linepithema humile* (Tsutsui and Case 2001), and *Sitobion avenae* (Figueroa et al. 2005).

Furthermore, it is reported that many invasive species exhibit reduced genetic variation as a result of founder effects and bottlenecks during colonization and establishment (Hawley et al. 2006; Lindholm et al. 2005; Tsutsui et al. 2000). New invasive populations are genetically less

Fig. 1 Unrooted neighbor-joining (NJ) phylogenetic consensus tree generated from the COI gene sequences of *T. absoluta* and from other insects (*Bombyx mori*, *Scrobipalpus sacculicola*, *Scrobipalpus tussilaginis*, *Gnorimoschema herbichii*, and *Gnorimoschema subterranean*) as the outgroup. The consensus tree was inferred from 2000 bootstrap replicates and only values >90% are shown at the internodes. The scale bar shows two nucleotide exchanges per 100 nucleotides

variable than the original population from which they are originated, because they are only a subset of the genetic diversity present in the native population (Nei et al. 1975; Chakraborty and Nei 1977; Cifuentes et al. 2011). Similarly, when introduced populations are small, there is a decreased genetic diversity (Roderick 2004). However, reduction in genetic diversity is generally harmful but occasionally contributed to the success of invasive species in some arthropods (Tsutsui and Case 2001; Figueroa et al. 2005). In *T. absoluta*, Cifuentes et al. (2011) ruled out the influence founder effects on the genetic homogeneity of the Mediterranean populations. Furthermore, genetic homogeneity in two continents (Europe and South America) was found in the mtCOI and rDNA analyses, suggesting that the Mediterranean populations are probable derived from a South American population or also the South American population is itself invasive (Cifuentes et al. 2011).

In conclusion, with the level of genetic variation of *T. absoluta* shown in the mtCOI marker in the present study, it is not possible to identify the exact origin of the Indian and

Nepal populations using the present marker. However, this is first attempt to generate molecular data for this new invasive species from India and Nepal. Furthermore, other genetic markers like microsatellites could be used in future studies to trace the exact route of origin of this destructive invasive pest for developing effective management strategies.

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Author contributions SPR collected and identified specimens. TS performed molecular work. Conceptualization: SPR and NMM. Data curation: SPR, TS, and SSS. Data analysis: SPR and ASRB. Supervision: SPR and KC. Funding acquisition: SPR. All authors read, reviewed and approved the manuscript.

Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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