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Some remarks on the genetic uniformity of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

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Abstract
Genetic variability of a lepidopteran pest was studied by investigating the mitochondrial DNA CO1 (5') of seven Tunisian populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). We have found a high genetic homogeneity which may confirm the hypothesis that this species was introduced only once in Tunisia. Alternatively, a selective sweep could be responsible for the absence of variability in the mitochondrial DNA.

Keywords: Pest, tomato, Solanaceae, crop, damage, barcode

1. Introduction

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae), is considered in Tunisia, since its first detection in 2008, as one of the most serious pest causing extensive damages mainly to tomato (*Solanum lycopersicum* L.) crop [6]. Other cultivated solanaceous plants including potato, (*S. tuberosum* L.), eggplant (*S. melongena* L.), sweet pepino (*S. muricatum* L.) and tobacco (*Nicotiana tabacum* L.) as well as wild plant species were also reported as secondary hosts for *T. absoluta* [9, 10, 12, 13, 15, 19, 21]. *Tuta absoluta*, which is a multivoltine non-diapausing species, was characterized by a high potential of growth performing several generations per year depending on climatic conditions and quality of existed hosts [8, 15]. This invasive pest can reduce significantly the tomato productions up to 80-100% in newly invaded areas both in protected and open fields if no control procedures are taken [1, 9]. Damages are caused by larvae which can feed on all aerial parts of tomato plants (leaves, buds, stem and fruits) by making large galleries [1, 18]. Secondary pathogens may invade later damaged fruits leading to their rot which can reduce the crop production and therefore inducing heavy cost losses to farmers [2, 9, 10, 11, 16]. Control programs based mainly on pesticides applications were carried out to manage this pest since its first detection in all production countries around the world [6]. Alternative pest management has been also investigated as sustainable tools used in IPM strategies deployed against this pest [5].

The study of genetic variability of *T. absoluta* is essential to establish an efficient IPM programs [3]. To identify possible genetic diversity which may have been developed by this invasive species molecular markers, such as AFLP and microsatellites markers were tested respectively for Brazilian and Tunisian populations [4, 20].

The aim of this work was to investigate if any genetic variation is expressed in Tunisian populations of *T. absoluta* using the standard DNA barcode (CO1) as a marker.

2. Material and methods

Infested tomato leaves with *Tuta* larvae were sampled from different regions located in Northern and Southern Tunisia as indicated in Table 1. Only L2 instars were preserved for DNA analysis. Second segment of larval thorax was placed into lysis plates containing one droplet of ethanol (96%) for DNA barcoding (cytochrome c oxidase subunit I, COI 5'). Three replicates were considered for each region resulting in a total of 21 individuals analyzed. All DNA analysis steps, including DNA extraction, PCR and DNA sequencing were carried out following standard high-throughput protocols [14] at the Canadian Centre for DNA Barcoding, Guelph, Canada (CCDB) (http://ccdb.ca/resources.php). Tunisian data were blasted against >100 other COI sequences of *T. absoluta* on BOLD Data Systems (Ratnasingham & Hebert 2007) using the identification engine of BOLD.

All data are accessible on BOLD in the public dataset DS-ABSOLUTA.

3. Results and discussion

A total of 18 barcodes sequences were obtained from 21 specimens of Tunisian *T. absoluta*. All sequences resulted belong to one single haplotype for all *T. absoluta* specimens regardless of their host-plants and localities. The Tunisian COI haplotype exactly corresponds to that of >100 examined COI barcodes including vouchers from countries like France, Netherlands, Portugal, Serbia, Bosnia and Hercegovina, Montenegro, Egypt, Saudi Arabia, Kenya, Tanzania, India. This uniformity in the mtDNA may indicate a genetic bottleneck and/or a selective sweep at a global level.

Our results are supported by those obtained by [7] which indicated high genetic homogeneity found in *T. absoluta* populations from the Mediterranean Basin and South America, based on mtCOI and ITS rDNA sequence analysis. Other studies on Tunisian *T. absoluta* using Randomly Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) technology on genomic DNA, however, resulted in a comparatively high genetic diversity as well as in a significant differentiation between populations [3].

Further research is needed to investigate the genetic variability for *T. absoluta* basing on different markers and involving larger samples from many locations and host-plants.

Table 1: Sampling data of collected larvae

Collection date	Province	Region	Host plant/site	Locality	Latitude	Longitude
18/12/2015	Tozeur	Mzara	Tomato/greenhouse	South-west	33°55.1808′N	8°8.0112′E
18/12/2015	Guebeli	Lymeguez	Tomato/greenhouse	South-west	33°42.2634′N	8°58.1418′E
17/12/2015	Gabes	Ben Guilouf	Tomato/greenhouse	South-East	33°52.8876′N	10°5.892′E
28/10/2015	Beja	Testour	Potato/open field	North-West	36°43.5384′N	9°10.9014′E
22/10/2015	Bizerte	Teskreya	Tomato/open field	North-west	37°16.4652′N	9°52.4346′E
16/10/2015	Nabeul	Bouargoub	Tomato/open field	North-East	36°27.3636′N	10°44.2578′E
07/10/2015	Ariana	Kalaat el Andalous	Tomato/open field	North-East	36°51.6072′N	10°11.6022′E

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