ONLINE RESOURCES

Development of polymorphic microsatellite loci for the tomato leaf miner, *Tuta absoluta* (Lepidoptera: Gelechiidae)

ASMA BETTAIBI[1], MAHA MEZGHANI-KHEMAKHEM[1]*, ZEINEB SOLTANI[1], HANEM MAKNI[1,2] and MOHAMED MAKNI[1]

[1]*Faculty of Sciences of Tunis, Research Unit on Genomics of Crop Insect Pests, University of Tunis El-Manar, Campus El Manar 2092 Tunis, Tunisia*
[2]*Higher Institute of Animation for Youth and Culture, University of Tunis, 2055 Bir El Bey, Tunisia*

[Bettaibi A., Mezghani-Khemakhem M., Soltani Z., Makni H. and Makni M. 2013 Development of polymorphic microsatellite loci for the tomato leaf miner, *Tuta absoluta* (Lepidoptera: Gelechiidae). *J. Genet.* **92**, e110–e112. Online only http://www.ias.ac.in/jgenet/OnlineResources/92/e110.pdf]

Introduction

The tomato leaf miner *Tuta absoluta* (Lepidoptera: Gelechiidae) is a devastating pest of tomato originating from South America (García and Espul 1982). Since its initial detection in tomato glasshouses in Spain at the end of 2006, *T. absoluta* has been spreading rapidly across Europe, northern Africa and Middle East, causing severe damage to tomato crops (Desneux *et al.* 2010; Roditakis *et al.* 2010; Abbes *et al.* 2012). The main host plant of *T. absoluta* is tomato (*Lycopersicon esculentum*). Nevertheless this pest also attacks various plants as secondary hosts such as potato (*Solanum tuberosum* L.), sweet pepper (*S. muricatum* L.), eggplant (*S. melongena* L.) and various wild solanaceous plants (Siqueira *et al.* 2000).

Variable molecular markers were used to estimate genetic diversity and population structure of this invasive species. AFLP markers were used to assess genetic diversity of *T. absoluta* populations from Brazil (Suinaga *et al.* 2004). Mitochondrial and nuclear markers were also used to estimate genetic diversity of *T. absoluta* populations from Spain (Cifuentes *et al.* 2011) and, more recently, RAPD markers have been applied to understand the structure of *T. absoluta* populations from Tunisia to furnish information about genetic variation during the colonization process (Bettaibi *et al.* 2012).

In recent years, microsatellites markers have proven to be very powerful in studying genetic structure of many insect populations such as *Myzus persicae* (MingHua *et al.* 2009); *Aphis spiraecola* (Cao *et al.* 2012) and *Bactrocera dorsalis* (Isasawin *et al.* 2012).

In the current study, we have developed microsatellite loci for *T. absoluta* from an enriched genomic library which may be helpful in the study of its population genetic structure during invasion.

Materials and methods

Total genomic DNA of *T. absoluta* was extracted using the cetyltrimethylammonium bromide (CTAB) protocol described by Doyle and Doyle (1987). Microsatellite loci were isolated following the method of (Glenn and Schable 2005). Genomic DNA was digested with the restriction enzyme *Rsa*I (Promega, Madison, USA) at 37°C overnight. The digestion products were ligated to double-stranded Super SNX24 linkers overnight at 16°C using T4 DNA ligase (New England Biolabs, UK). The ligation products were then amplified by PCR with super SNX24F as primer (5'-GTTTAAGGCCTAGCTAGCAGAATC-3') for 20 cycles of 20 s denaturation at 94°C, 20 s annealing at 60°C and 1 min extension at 72°C. Genomic DNA fragments containing microsatellite loci were captured by hybridization with $(AG)_{12}$, $(TG)_{12}$ and $(CAA)_8$ biotin-labelled probes. The enriched DNA fragments were then amplified by PCR using super SNX24F as primer. PCR products were ligated to a pGEM T-easy vector (Promega, Madison, USA) and cloned in *Escherichia coli* DH5α strain (NEB, UK). Approximately, 324 recombined clones were revealed by blue-white screening on X-GAL/IPTG/ampicillin-LB agar plates. PCR using T7/SP6 primers showed 192 positive clones with an insert length between 500 and 1000 bp. After miniprep purification using the QIA Prep Plasmid DNA kit (Qiagen, USA), clones were selected and sequenced with T7 primer. Thirty-six sequences containing 15 microsatellite loci were obtained and submitted to the software Primer3 (Rozen and Skaletsky 2000) to define primers useful for screening polymorphism.

*For correspondence. E-mail: mahakm@planet.tn.

Keywords. enriched genomic library; microsatellite; *Tuta absoluta*.

Table 1. Characteristics of the eight polymorphic microsatellite loci isolated in *Tuta absoluta*.

Locus name	Repeat motif	Primer sequences (5' 3')	T_a (°C)	Product length (bp)	N	H_E	H_O	P (SB)	Null allele frequency estimate	GenBank accession number
TE6	$(TG)_{14}$	F: 5'CGAAAAAGTGAAAGCAAGGAC3' R: 5'TCCTCATTTAACATTCTGAGGTTTT3'	55	156	4	0.697	0.550	0.935	0.118	KC493810
TF3	$(AC)_{16}$	F: 5'TCGGGAAAATGAAAATGTCAC3' F: 5'GCCTAGCTAGCAGAATCACG3'	55	168	6	0.796	0.700	0.995	0.064	KC493811
TE2	$(GTT)_6$	F: 5'GAATCCCAAGTTTACCGCCC3' R: 5'ATTCCCGTAAACTTGGAAACA3'	60	170	3	0.650	0.825	0.017	−0.117	KC493813
TXE5	$(TACA)_6$	F: 5'TCGCAAGCTTTTCAAATCAA3' R: 5'TCTGACACCAGGGTTCATCC3'	55	166	6	0.814	0.950	0.012	−0.114	KC493816
TA27	$(TG)_{22}$	F: 5'CGTAAGGGAAATAGGCGTGA3' R: 5'AGCAGGCGTAGACCTTGGTA3'	60	168	6	0.765	0.950	0.001	−0.107	KC493817
TA23	$(CAA)_7$	F: 5'CGACAACGGAAGTGTCATGT3' R: 5'ACGTCAAATCGCATGGAAA3'	60	158	3	0.573	0.525	0.641	0.043	KC493818
TA16	$(GTAT)_5$	F: 5'CTTACCATCCACCACCAAGG3' R: 5'ATTTGCGCAATCCCAGATAG3'	60	155	2	0.499	0.375	0.977	0.209	KC493819
TA19	$(GTTT)_4$	F: 5'AACTTAACCATTCAACTGATCAACA3' R: 5'GGTCTAGTAGTTTTGGAGCCTGT3'	60	157	2	0.624	0.650	0.269	−0.020	KC493821

T_a, annealing temperature, N, observed number of alleles, H_O, observed heterozygosity, H_E, expected heterozygosity;
SB, sequential Bonferroni threshold at the significance level 0.05.

Fourteen individuals of *T. absoluta* collected from different sites located in the north (Manouba, Takelsa and Brij), centre (Kairouan, Chott Mariem and sfax) and south (Gabès, Kébili, Tozeur and Rjim Maatoug) of Tunisia were used to identify polymorphic loci. PCR was performed in a 25 μL mixture containing 20 ng of genomic DNA, 100 μM dNTPs, 10 μM of each primer, 5 μL of 5× buffer and 0.25 U *GoTaq* polymerase (Promega, Madison, USA). PCR conditions were as follows: initial denaturation at 94°C for 5 min followed by 35 cycles of 95°C for 1 min, annealing temperature of each locus for 1 min and extension at 72°C for 1 min, with a final extension at 72°C for 10 min. PCR products were revealed on 8% polyacrylamide denaturing gels under UV lights after ethidium bromide staining. The microsatellite alleles were sized by reference to the molecular marker 10 (Euromedex, France).

For each polymorphic locus, we calculated observed heterozygosity (H_O) and expected heterozygosity (H_E) using the program GENETIX ver. 4.05 (Belkhir *et al.* 2004). Deviation from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were calculated using GenePop ver. 4.2 (Raymond and Rousset 1995). Loci were further analysed in Mircro-Checker Software (Van Oosterhout *et al.* 2004) to test genotyping errors and to estimate the frequency of null alleles.

Results and discussion

A total of 36 clones were found to contain microsatellite motifs. After discarding sequences with few repeat regions and those not suitable for designing primers, 15 sequences were selected. Finally, eight polymorphic loci were isolated and characteristics of each locus are shown in table 1. Levels of polymorphism were tested on 40 individuals of *T. absoluta* collected on tomato from several regions in Tunisia.

The number of alleles per locus ranged from 2 to 6. The expected (H_E) and observed (H_O) heterozygozity varied from 0.499 to 0.814 and 0.375 to 0.950, respectively (table 1).

Following the sequential Bonferroni correction ($P < 0.05$, adjusted value), significant deviations from HWE associated with excess heterozygote were detected in three loci: TE2, TXE5 and TA27. The eight loci were also analysed to test for the presence of null alleles. Results indicated the presence of null alleles for the locus TA16 which may explain homozygous excess. There was no significant LD among pairs of these loci after Bonferroni correction, therefore allelic variations at these loci were considered independent.

In our study, six of the eight polymorphic microsatellites are generally informative to study genetic structure and to estimate gene flow of the invasive tomato leaf miner *Tuta absoluta* populations.

References

Abbes K., Harbi A. and Chermiti B. 2012 The tomato leafminer *Tuta absoluta* (Meyrick) in Tunisia: current status and management strategies. *EPPO Bull.* **2**, 226–233.

Belkhir K., Borsa P., Chikhi L., Raufaste N. and Bonhomme F. 2004 GENETIX 4.0.5.2., software 109 under Windows^{TM} for the genetics of the populations. Laboratory Genome, Populations, 110 Interactions, CNRS UMR 5000, University of Montpellier II, Montpellier, France.

Bettaibi A., Mezghani K. M., Bouktila D., Makni H. and Makni M. 2012 Genetic variability of the tomato leaf miner (*Tuta absoluta* Meyrick; Lepidoptera: Gelechiidae), in Tunisia, inferred from RAPD-PCR. *Chilean J. Agric. Res.* **2**, 212–216.

Cao J. L. J., Niu J., Liu X. and Zhang Q. 2012 Population structure of *Aphis spiraecola* (Hemiptera: Aphididae) on pear trees in China identified using microsatellites. *J. Economic Entomol.* **2**, 583–591.

Cifuentes D., Chynoweth R. and Bielza P. 2011 Genetic study of Mediterranean and SouthvAmerican populations of tomato leafminer *Tuta absoluta* (Povolny, 1994) (Lepidoptera: Gelechiidae) using ribosomal and mitochondrial markers. *Pest Manag. Sci.* **67**, 1155–1162.

Desneux N., Wajnberg E., Wyckhuys K. A. G., Burgio G., Arpaia S., Narváez-Vasquez C. A. *et al.* 2010 Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. *J. Pest Sci.* **83**, 197–215.

Doyle J. J. and Doyle J. L. 1987 A rapid DNA isolation procedure for small quantities of fresh leaves tissue. *Phytochem. Bull.* **19**, 11–15.

García M. F. and Espul J. C. 1982 Bioecology of the tomato moth (*Scrobipalpula absoluta*) in Mendoza, Argentine Republic. *Revista de Investigaciones Agropecuarias.* **17**, 135–146.

Glenn T. C and Schable N. A. 2005 Isolating microsatellite DNA loci. *Methods Enzymol.* **395**, 202–222.

Isasawin S., Aketarawong N. and Thanaphum S. 2012 Characterization and evaluation of microsatellite markers in a strain of the oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae), with a genetic sexing character used in sterile insect population control. *Eur. J. Entomol.* **3**, 331–338.

MingHua H., YongMo W., Zhe W. and ZuoRui S. 2009 Genetic differentiation of *Myzus persicae* based on host plants revealed by using microsatellites. *Chin. Bull. Entomol.* **2**, 244–249.

Raymond M. and Rousset F. 1995 Genepop: Population genetics software for exact tests and ecumenicism. *J. Hered.* **86**, 248–249.

Roditakis E., Papachristos D. and Roditakis N. E. 2010 Current status of the tomato leafminer *Tuta absoluta* in Greece. *EPPO Bull.* **40**, 163–166.

Rozen S. and Skaletsky H. 2000 Primer3 on the WWW for General Users and for Biologist Programmers. In *Bioinformatics methods and protocols* (ed. S. Krawetz and S. Misener), pp. 365–386. Humana Press, Totowa, USA.

Siqueira H. A., Guedes R. N. and Picanco M. C. 2000 Insecticide resistance in populations of *Tuta absoluta* (Lepidoptera: Gelechiidae). *Agric. For. Entomol.* **2**, 147–153.

Suinaga F. A., Casali V. W. D., Picanço M and Foster J. 2004 Genetic divergence among tomato leafminer populations based on AFLP analysis. *J. Pesq. Agropec. Bras.* **7**, 645–651.

Van Oosterhout C., Hutchinson W. F., Wills D. P. M and Shipley P. 2004 MicroChecker: Software foridentifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* **4**, 535–538.

Received 29 April 2013, in final revised form 19 August 2013; accepted 22 August 2013
Published on the Web: 10 December 2013