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Genetic divergence among tomato leafminer populations based on AFLP analysis

Fábio Akiyoshi Suinaga(1), Vicente Wagner Dias Casali(2), Marcelo Picanço(3) and John Foster(4)

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Abstract – The objective of this work was to determine the genetic differences among eight Brazilian populations of the tomato leafminer Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae), from the states of Espírito Santo (Santa Tereza), Goiás (Goianápolis), Minas Gerais (Uberlândia and Viçosa), Pernambuco (Camocim de São Félix), Rio de Janeiro (São João da Barra) and São Paulo (Paulínia and Sumaré), using the amplified fragment length polymorphism (AFLP) technique. Fifteen combinations of EcoRI and MseI primers were used to assess divergence among populations. The data were analyzed using unweighted pair-group method, based on arithmetic averages (UPGMA) bootstrap analysis and principal coordinate analysis. Using a multilocus approach, these populations were divided in two groups, based on genetic fingerprints. Populations from Goianápolis, Santa Tereza, and Viçosa formed one group. Populations from Camocim de São Félix, Paulínia, São João da Barra, Sumaré, and Uberlândia fitted in the second group. These results were congruent with differences in susceptibility of this insect to insecticides, previously identified by other authors.

Index terms: Lycopersicon esculentum, biometry.

Introduction

The tomato leafminer, Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae), is an oligophagous pest which damages eggplants, green peppers, and especially tomatoes. It is one of the most important insect pests of tomato in the Neotropical region, mainly in South America (Picanço et al., 1998). T. absoluta is also a pest of economic importance in Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Peru, Uruguay and Venezuela (Picanço et al., 1997).
In order to control this pest, farmers perform more than 36 insecticide applications within a single crop cycle. As a consequence, reduced efficiency and control failure of the insecticides used against *T. absoluta* have been reported in Brazil (Guedes et al., 1994; Siqueira et al., 2000a) and in Chile (Salazar & Araya, 1997). Siqueira et al. (2000b) showed differences among Brazilian populations of *T. absoluta*, in susceptibility to abamectin, cartap, methamidophos, and permethrin, which could indicate a development of resistance of this insect to these insecticides as stated by Siqueira et al. (2000a, 2000b). Despite these facts, there has been no investigation of molecular divergence among these populations.

A recently developed technique for assessing molecular differences among populations is the amplified fragment length polymorphisms (AFLP) (Vos et al., 1995). Because of the vast number of loci available, AFLP has a great potential to discover genetic divergence (McMichael & Prowell, 1999). Besides, this technique has demonstrated to be a sensitive and powerful tool to distinguish among genotypes from different geographic origins as well as providing sufficient molecular markers for characterizing insect genomes (Parsons & Shaw, 2001). Several examples support the use of AFLP for genetic studies of insect populations such as: *Spodoptera frugiperda* (J.E. Smith) (McMichael & Prowell, 1999), *Lymantria dispar* (Linnaeus) (Reineke et al., 1999), and *Bemisia tabaci* (Gennadius) (Cervera et al., 2000).

The objective of this work was to determine genetic differences among eight populations of *Tuta absoluta* by the amplified fragment length polymorphism technique.

### Material and Methods

#### Insect populations

Eight populations of *T. absoluta* from the states of Espírito Santo (Santa Tereza), Goiás (Goiâniapolis), Minas Gerais (Uberlândia and Viçosa), Pernambuco (Camocim de São Félix), and São Paulo (Paulínia and Sumaré), Brazil, were used in this study. All populations, except Sumaré, were resistant to abamectin, cartap, methamidophos, and permethrin (Siqueira et al., 2000b). Colonies of *T. absoluta* were initially established by at least 500 larvae obtained from heavily infested plants, collected at each sampling site from tomato field crops. Each population was reared isolated from the others, on tomato plants of Santa Clara variety, without insecticide exposure, enclosed in cages and maintained in the laboratory and previously tested for resistance to abamectin, cartap, methamidophos, and permethrin by Siqueira et al. (2000b) (Table 1). All samples were composed of 4th instar larvae and were fixed and stored in 70% ethanol.

#### DNA isolation

DNA was extracted from fifteen individual larvae per population, using the entire body, following cetyltrimethylammonium bromide (CTAB) protocol (Reineke et al., 1998). DNA pellets were dissolved in 20 μL of TE buffer and short term stored at 4°C. The quantity of DNA in each preparation was estimated by electrophoresis of 1 μL of each suspension on 0.8% agarose gels containing 0.2 μL/mL of ethidium bromide and then comparing band intensity with known quantities of lambda phage DNA.

#### AFLP analysis

Templates for amplified fragment length polymorphism (AFLP) reactions were prepared following Vos et al. (1995), using approximately 200 ng of genomic DNA for restriction digests with the endonucleases EcoRI (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA) and MseI (New England Biolabs, Beverly, MA, USA) and ligation of adapters (Table 2). The restriction-ligation mixtures (RLM) were diluted 1:10 in TE buffer and these products served as templates for further pre-amplification reactions. The reaction volumes were 25.5 μL, and composed of 2.5 μL of each RLM, 0.5 μL of AmpliTaq DNA Polymerase (Applied Biosystems, Foster City, CA, USA), 20 μL of AFLP Pre-Amp Primer Mix II, 10 μL of ddH₂O, and 2 μL of AmpliTaq DNA Polymerase (Applied Biosystems, Foster City, CA, USA). The reaction mixtures were subjected to 16 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 10 min, with a final extension step at 72°C for 30 min. The amplified products were separated on 3% agarose gels, visualized under UV light, and photographed.

### Table 1. Origin of Tuta absoluta populations, reared in laboratory, used in this work.

<table>
<thead>
<tr>
<th>Code</th>
<th>Country</th>
<th>State</th>
<th>Insecticide resistance status</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO</td>
<td>Goiâniapolis</td>
<td>Goiás</td>
<td>2</td>
</tr>
<tr>
<td>PA</td>
<td>Paulínia</td>
<td>São Paulo</td>
<td>3</td>
</tr>
<tr>
<td>PE</td>
<td>Camocim de São Félix</td>
<td>Pernambuco</td>
<td>2</td>
</tr>
<tr>
<td>SJ</td>
<td>São João da Barra</td>
<td>Rio de Janeiro</td>
<td>3</td>
</tr>
<tr>
<td>ST</td>
<td>Santa Tereza</td>
<td>Espírito Santo</td>
<td>2</td>
</tr>
<tr>
<td>SU</td>
<td>Sumaré</td>
<td>São Paulo</td>
<td>4</td>
</tr>
<tr>
<td>UB</td>
<td>Uberlândia</td>
<td>Minas Gerais</td>
<td>2</td>
</tr>
<tr>
<td>VC</td>
<td>Viçosa</td>
<td>Minas Gerais</td>
<td>4</td>
</tr>
</tbody>
</table>

1) Resistant to at least two, three or four insecticides. 2) Data not available.
(Life Technologies, Gaithersburg, MD, USA), containing primers E0 and MC (Table 2), and 2.5 l of 10 X PCR buffer plus MgCl₂ (Applied Biosystems). Polymerase chain reactions (PCRs) amplifications were performed in an applied biosystems 9700 thermalcycler using 20 cycles. Each cycle comprised 30 seconds at 94°C, 1 minute at 56°C, and 1 minute at 72°C. Selective amplifications were done using various combinations of EcoRI and MseI primers, both of them with three selective nucleotides, E+3 and M+3 (Table 2). A total reaction volume (10 l) per template-primer set was composed of 2 l of 1:100 pre-amplified template DNA, 2 l MseI primer containing dNTP (Life Technologies), 0.06 l of AmpliTaq DNA Polymerase (Applied Biosystems). PCR amplifications consisted of one cycle of 30 seconds at 94°C, 30 seconds at 65°C, and 1 minute at 72°C; 12 cycles in which all denaturing and extending temperatures remained the same, and the annealing temperature was decreased by 0.7°C in each cycle; finally 23 cycles of 30 seconds at 94°C, 30 seconds at 56°C, and 1 minute at 72°C were performed.

**Detection and scoring of AFLP fragments**

After selective amplification, reactions were stopped with 5 l of gel loading buffer (consisting of 95% deionized formamide, 20 mM EDTA pH 8.0 and 1 mg/ml bromophenol blue). This mixture was heated at 94°C for 3 minutes, then quickly cooled on ice prior to gel loading. AFLP products were resolved on denaturing gels containing 6.5% polyacrylamide (Li-Cor, Lincoln, NE, USA), 1.2 l of 10 X PCR buffer plus MgCl₂ (Applied Biosystems), and 0.06 l of AmpliTaq DNA Polymerase (Applied Biosystems). PCR amplifications consisted of one cycle of 30 seconds at 94°C, 30 seconds at 65°C, and 1 minute at 72°C; 12 cycles in which all denaturing and extending temperatures remained the same, and the annealing temperature was decreased by 0.7°C in each cycle; finally 23 cycles of 30 seconds at 94°C, 30 seconds at 56°C, and 1 minute at 72°C were performed.

**Data analysis**

Genetic similarities among AFLP fingerprints from each population were estimated according to the formula of Dice (1945), GS_{ij} = 2a/(2a + b + c), where GS_{ij} is the genetic similarity between individuals i and j; a is the number of polymorphic bands that are shared by i and j; b is the number of bands present in i and absent in j; and c is the number of bands present in i and absent in j; and c is the opposite of b. Genetic relationships among AFLP fingerprints were represented in a dendrogram based on the unweighted pair-group method of arithmetic averages (UPGMA) with bootstrapping (500 replicates) and a principal coordinate analysis bidimensional scatter plot, respectively done by treecon for windows software package version 1.3b (Van de Peer & De Wachter, 1994) and NTSYS-PC version 2.02k (Rohlf, 1997).

**Results and Discussion**

Seven primer combinations were used for the analysis of populations of *T. absoluta*. Considering these combinations, fifteen amplified loci were selected for which the most common allele in each population occurred at a frequency greater than or equal to 75% (Table 3). Twenty-eight different multilocus AFLP fingerprints were identified in the 32 *T. absoluta* sampled (Table 4).
from Paulínia; ST 1 to 4: four fingerprints from Santa Tereza; SU 1 to 4: four fingerprints from Sumaré; UB 1 to 4: four fingerprints from Uberlândia; Viçosa (VC 1 to 3); and São João da Barra (SJ 1 to 3). The results of this work partially corroborated previous information (Siqueira et al., 2000b) regarding genetic differences related to insecticide resistance among T. absoluta populations. The separation of 28 AFLP fingerprints of this insect, in two distinct groups, was supported not only by UPGMA bootstrap analysis but also by principal coordinate analysis. According to Siqueira et al. (2000a, 2000b), tomato leafminer larvae from Paulínia, São João da Barra, and Uberlândia (Figure 1) were more susceptible to abamectin than populations from Viçosa, Lavras, and Araguari. These studies indicated differences in the resistance levels among different populations for each tested insecticide. Such variability suggests differential selection pressures, genetic diversity in the resistance mechanisms among the insect populations, or both (Kerr & Gaylor, 1992). This fact could explain the formation of different groups (A and B) of T. absoluta populations used in the present work.

The accumulated variance of the first three eigenvalues, generated by the principal coordinate analysis was greater than sixty percent. As suggested

<table>
<thead>
<tr>
<th>Primer pair combinations (bp)</th>
<th>GO</th>
<th>PA</th>
<th>PE</th>
<th>SU</th>
<th>ST</th>
<th>UB</th>
<th>VC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. MCTT-EACT (73)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>2. MCTT-EACT (135)</td>
<td>1 (0)</td>
<td>0.75 (1)</td>
<td>0.75 (1)</td>
<td>1 (0)</td>
<td>0.75 (1)</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>3. MCAT-EACA (174)</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>1 (1)</td>
<td>1 (0)</td>
<td>0.75 (0)</td>
<td>0.75 (1)</td>
</tr>
<tr>
<td>4. MCTG-EACA (189)</td>
<td>1 (0)</td>
<td>0.75 (1)</td>
<td>0.75 (1)</td>
<td>0.75 (1)</td>
<td>0.75 (1)</td>
<td>0.75 (1)</td>
<td>0.75 (1)</td>
</tr>
<tr>
<td>5. MCTG-EACA (208)</td>
<td>1 (0)</td>
<td>1 (1)</td>
<td>0.75 (0)</td>
<td>0.75 (1)</td>
<td>0.75 (0)</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>6. MCTT-EACT (209)</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>1 (1)</td>
<td>0.75 (1)</td>
<td>1 (1)</td>
<td>0.75 (1)</td>
<td>0.75 (1)</td>
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<tr>
<td>7. MCTT-EAGG (213)</td>
<td>1 (1)</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>0.75 (1)</td>
<td>1 (0)</td>
<td>1 (1)</td>
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<tr>
<td>8. MCTC-EAGG (219)</td>
<td>1 (1)</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>0.75 (0)</td>
<td>1 (0)</td>
<td>1 (0)</td>
</tr>
<tr>
<td>9. MCTG-EACA (242)</td>
<td>1 (0)</td>
<td>0.75 (1)</td>
<td>1 (1)</td>
<td>0.75 (0)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>10. MCAF-EACB (266)</td>
<td>1 (1)</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>1 (1)</td>
<td>0.75 (0)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>11. MCTT-EAGG (306)</td>
<td>0.75 (1)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>1 (0)</td>
<td>0.75 (1)</td>
<td>1 (1)</td>
<td>0.75 (1)</td>
</tr>
<tr>
<td>12. MCTT-EACT (304)</td>
<td>1 (0)</td>
<td>1 (1)</td>
<td>0.75 (1)</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>1 (0)</td>
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<tr>
<td>13. MCTA-EAGG (339)</td>
<td>0.75 (0)</td>
<td>0.75 (0)</td>
<td>0.75 (1)</td>
<td>0.75 (1)</td>
<td>1 (0)</td>
<td>0.75 (1)</td>
<td>1 (1)</td>
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<tr>
<td>14. MCAF-EACT (391)</td>
<td>1 (1)</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>1 (1)</td>
<td>1 (0)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>15. MCAF-EACB (426)</td>
<td>1 (1)</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>1 (0)</td>
<td>1 (1)</td>
<td>1 (0)</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

(a) Numbers inside the brackets show the status of the character absent (0) or present (1).

Table 3. Frequency of occurrence of most common allele on fifteen loci selected by amplified fragment length polymorphism (AFLP) technique for Tuta absoluta populations identification (1).

Table 4. Presence (1) or absence (0) of amplified fragment length polymorphism (AFLP) fingerprints in Tuta absoluta. (ST1 to 3), Sumaré (SU1 to 3), and Uberlândia (UB1 to 3).

The dendrogram, based on unweighted pair-group method, separated the AFLP fingerprints in two distinct groups (Figure 1). The first (group A) was composed of multilocus data of larvae from Camocim de São Félix, Paulína, Camocim de São Félix, Paulína, Goianápolis, Santa Tereza, and Viçosa. Although bootstrap values showed limited support for some nodes, the same type of cluster was observed (Figure 2).

The results of this work partially corroborated previous information (Siqueira et al., 2000b) regarding genetic differences related to insecticide resistance among T. absoluta populations. The separation of 28 AFLP fingerprints of this insect, in two distinct groups, was supported not only by UPGMA bootstrap analysis but also by principal coordinate analysis. According to Siqueira et al. (2000a, 2000b), tomato leafminer larvae from Paulínia, São João da Barra, and Uberlândia (Figure 1) were more susceptible to abamectin than populations from Viçosa, Lavras, and Araguari. These studies indicated differences in the resistance levels among different populations for each tested insecticide. Such variability suggests differential selection pressures, genetic diversity in the resistance mechanisms among the insect populations, or both (Kerr & Gaylor, 1992). This fact could explain the formation of different groups (A and B) of T. absoluta populations used in the present work.

The accumulated variance of the first three eigenvalues, generated by the principal coordinate analysis was greater than sixty percent. As suggested

(a) Numbers inside the brackets show the status of the character absent (0) or present (1). (2) GO: Goianápolis; PA: Paulínia; PE: Camocim de São Félix; SJ: São João da Barra; ST: Santa Tereza; SU: Sumaré; UB: Uberlândia; VC: Viçosa.
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Figure 1. Dendrogram of eight populations of *Tuta absoluta* amplified fragment length polymorphism (AFLP) fingerprints resulting from unweighted pair group method (UPGMA) with cluster analysis. Bootstrap support (percent of 500 replicates) is indicated for each branch. GO1 to 3: three fingerprints from Goiânia; PE1 to 3: three fingerprints from Camocim de São Félix; SJ1 to 3: three fingerprints from São João da Barra; VC1 to 3: three fingerprints from Viçosa; PA1 to 4: four fingerprints from Paulínia; ST1 to 4: four fingerprints from Santa Tereza; SU1 to 4: four fingerprints from Sumaré; UB1 to 4: four fingerprints from Uberlândia.

Figure 2. Scatter plot of principal coordinate analysis of 28 amplified fragment length polymorphism (AFLP) fingerprints of *Tuta absoluta*. Numbers in the parenthesis, located in the X and Y axis, indicate cumulative percentages of the eigenvalues. Group A: PE1 to 3: three fingerprints from Camocim de São Félix; SJ1 to 3: three fingerprints from São João da Barra; PA1 to 4: four fingerprints from Paulínia; SU1 to 4: four fingerprints from Sumaré; UB1 to 4: four fingerprints from Uberlândia. Group B: GO1 to 3: three fingerprints from Goiânia; VC1 to 3: three fingerprints from Viçosa; ST1 to 4: four fingerprints from Santa Tereza.
by Sparks et al. (1999), when the accumulated variance of the first three eigenvalues is greater than 80%, it is reasonable to study the characteristics (in this case population divergence) in a bidimensional space rather than an n-dimensional one. In this work, the first and the third coordinate axes were plotted in order to show the best dispersion of these fingerprints. The same trend displayed on the UPGMA dendrogram (Figure 1) was observed in the plot of the two principal coordinate axes (Figure 2), that is, the division of twenty-eight AFLP fingerprints into two groups described.

The differential selection pressures could be obtained by using different compounds, over-recommended insecticide dosages, as well as frequency of application. Even though the insecticide registration in Brazil is a national policy (Andrei, 1999), there is a variation in the acceptance or usage of a particular product in a specific region (Guedes et al., 1995). For example, in the region of Goiânia, despite the low efficiency in controlling T. absoluta, farmers continuously spray methamidophos against this pest. As a result of this practice, failure in control is detected and an increase of the insecticide dosage or a raise in the frequency of applications is often observed (Picanço et al., 1995). In this case, agricultural practices are promoting the selection of resistant individuals of T. absoluta to insecticides, but with different—morphological, physiological and ecological—characteristics depending on the origin of the insects, and in part explaining the results found in this work.

Besides the effects of the insecticides on T. absoluta strains, alternative hosts could generate differential preferences for acceptance between within population(s) (Jaenike, 1990). Genetic based differences in host acceptance have been found between US mainland and Virgin Island populations of Halitosis viruses (Lepidoptera: Noctuidae) (Schneider & Roush, 1986; Waldvogel & Gould, 1990). Additionally, Moreira et al. (2001) working with populations of T. absoluta from Camocim de São Félix, Santa Tereza, Uberlândia, and Vçosa observed differences on biological characteristics of this insect when feeding on several wild tomato species. The authors also proposed the division of these populations in two groups, i.e., Santa Tereza and Uberlândia, presented in group B and A, respectively, in order to obtain a core collection of this insect. All of these results could support the results found in this work.

**Conclusions**

1. AFLP technique demonstrates usefulness in the study of geographical variation of T. absoluta populations.

2. The results obtained can be used to explain or to detect the differences in the populations responses to insecticides, as well as host plants.

3. Data obtained can also be used in the breeding programs for tomato resistance to T. absoluta, supporting the usage of one representative population, depending on the site of plant selection.

**References**


Genetic divergence among tomato leafminer populations


