COMPARATIVE ANALYSIS OF THE DELTOCEPHALINE LEAFHOPPER TRANSCRIPTOMES FOR THE ELUCIDATION OF VECTOR COMPETENCE GENES

Christian Ayala-Ortiz\textsuperscript{a}, Trenna Blagden\textsuperscript{a}, Chris Dietrich\textsuperscript{b}, Dmitry Dmitriev\textsuperscript{b}, and Astri Wayadande\textsuperscript{a}

\textsuperscript{a}Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK
\textsuperscript{b}Illinois Natural History Survey, Champaign-Urbana, IL

BACKGROUND

- The subfamily Deltocephalinae (Hemiptera: Cicadellidae) is the largest and more diverse subfamily of Deltocephalinae [1].
- Most of the leafhopper species that are vectors of economically important diseases are included within this subfamily [2].
- Even though insect-borne plant pathogens cause serious damage to economically important crops, the mechanisms that regulate their transmission process are not completely understood [3].
- It is believed that the ability to effectively acquire and transmit a pathogen may be genetically regulated in the insect vectors [5].
- Therefore, the comparison of the transcriptomes of vector and non-vector leafhoppers can produce new insights regarding this process.

OBJECTIVE

- Assemble, annotate and compare the transcriptomes of the six leafhopper species to identify candidate genes involved in vector competence

MATERIALS AND METHODS

- RNA extraction
  Total RNA of the six leafhoppers was extracted using the E.Z.N.A. Mollusk RNA kit (Omega Bio-Tek, Inc., Norcross, GA). Some insects were recollected from the field during late summer and early fall, while others come from colonies maintained at Oklahoma State University.
- Transcriptome sequencing and assembly
  Transcriptomes were sequenced using an Illumina HiSeq 4000 platform. Transcriptome assembly was made using the Trinity de novo pipeline [6]. Duplicates with more than 95% similarity were removed to obtain “unigene” sets for each species.
- Annotation and clustering analysis
  Peptide sequences were obtained with TransDecoder [7]. CD-HIT [8] was used to perform a clustering analysis of the peptides.

RESULTS

Table 1. Trinity assembly and unigene set statistics

<table>
<thead>
<tr>
<th>Species</th>
<th>Trinity assembly</th>
<th>Unigene set</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># contigs N50 (bp)</td>
<td># contigs N50 (bp)</td>
</tr>
<tr>
<td>B. neglecta</td>
<td>542000 1365</td>
<td>344753 1323</td>
</tr>
<tr>
<td>B. rubrostriata</td>
<td>415639 1564</td>
<td>285542 1416</td>
</tr>
<tr>
<td>D. maidis</td>
<td>221586 2020</td>
<td>133542 2000</td>
</tr>
<tr>
<td>E. exitiosus</td>
<td>336694 1599</td>
<td>178231 1578</td>
</tr>
<tr>
<td>G. nigrifrons</td>
<td>287140 1564</td>
<td>178157 1647</td>
</tr>
<tr>
<td>M quadrilineatus</td>
<td>391269 1452</td>
<td>253294 1375</td>
</tr>
</tbody>
</table>

Table 2. Percentage of transcripts annotated to different biological databases

<table>
<thead>
<tr>
<th>Species</th>
<th>Swiss-Prot</th>
<th>KEGG</th>
<th>GO</th>
<th>eggNOG</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. neglecta</td>
<td>44.96%</td>
<td>30.02%</td>
<td>33.80%</td>
<td>28.95%</td>
</tr>
<tr>
<td>B. rubrostriata</td>
<td>30.03%</td>
<td>17.34%</td>
<td>20.26%</td>
<td>16.74%</td>
</tr>
<tr>
<td>D. maidis</td>
<td>47.25%</td>
<td>28.98%</td>
<td>32.91%</td>
<td>28.25%</td>
</tr>
<tr>
<td>E. exitiosus</td>
<td>28.32%</td>
<td>16.54%</td>
<td>19.58%</td>
<td>15.91%</td>
</tr>
<tr>
<td>G. nigrifrons</td>
<td>41.90%</td>
<td>23.55%</td>
<td>27.46%</td>
<td>22.88%</td>
</tr>
<tr>
<td>M quadrilineatus</td>
<td>26.54%</td>
<td>17.12%</td>
<td>20.23%</td>
<td>16.50%</td>
</tr>
</tbody>
</table>

CONCLUSIONS

- Between 29.95% to 47.78% of all the transcripts produced a match to at least one of the tested functional databases.
- A total of 925 peptide sequences shared among vector species have less than 85% similarity to sequences obtained from non-vector leafhoppers.
- Several of those peptide sequences seems to be related with insect vector transmission and need to be further studied to have a better understanding of vector competence

ACKNOWLEDGEMENTS

- National Science Foundation; GoLife Project
- NIMFFAB
- Oklahoma State University Agricultural Experiment Station

REFERENCES


Figure 1. Clustering analysis of the peptide sequences. (A) Diagram of how the comparison was made. (B) Number of peptide sequences obtained in each group.

Figure 2. Five most represented GO terms of each domain in the vector peptide dataset and some associated insect vector transmission proteins.