Grape LeafRoll-associated Virus: A systems biology approach to understand Plant-Pathogens interaction in fruit ripening

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**Symptomatology of the Grape Leafroll Disease**

- In general, GLD symptoms do not appear until the crop advances toward the ripening onset.
- Disease with asymptomatic and symptomatic phases.
- Virus-host interactions modulated in a developmental stage-specific manner.

**Biological consequences**

- In leaf, changes in photosynthesis, carbohydrate metabolism, and flavonoid pathway (Gutha et al., 2010).

- In leaf, Nt photosynthesis and Chla fluorescence repressed during post-ripening stages (Endeshaw et al., 2014).

- In infected berries, dramatic alteration in the expression of photosynthesis, flavonoid and sugar transport-related genes (Expinosa et al., 2007, Vega et al., 2011).

**Downstream Analyses:**

**Comparison 1**

- NGS Sequencing - RNAseq approach through high definition mass spectrometry (Uniprot database)

**Comparison 2**

- MS Spectrometry - Unbiased Metabolomics

**Objectives:**

- In comparison # 1, we want to evaluate the impact of the infection on the transcriptional state at different times of the ripening.

- In comparison # 2, we want to determine the impact of the virus on the reduction of intra-cluster variability during the symptomatic period.

**Economic impact of Grapevine Leafroll Disease**

- Grape leaf roll disease (GLD) remains the most economically important viral disease across many wine-producing regions in the world.

- GLRD can have devastating effects on yield (up to 30-70% reduction) and grape quality (ripening delay, reduced accumulation of sugars, increased acidity, reduced tannin content and anthocyanin and undesirable flavor).

- Economic loss can range from $29,902 to $226,405/ha (Gomez et al., 2016).

**Biology of Grapevine Leafroll-Associated Viruses**

- Eleven viruses designated serially as GLRaV-1, -2, -3, etc.

- The taxonomy recognizes five GLRaV species in three genera (Ampelovirus, Clustrovirus, and Velirivirus).

- Several species of mealybugs and scale insects in the Ampelovirus. No vectors identified yet for GLRaV-2 and 7.

- Vast majority of genetic diversity were concentrated on the most economically important GLRaV-3.

**Insights on the Host-Virus interactions:**

- Virus-induced host RNA silencing and viral suppressors of host RNA silencing.

- Alteration of the host mRNA regulatory pathways:

  - In several crops, increasing evidence suggests that the virus infection triggers miRNAs production (Pradhan et al., 2015; Liu et al., 2015; Yin et al., 2013).

- Research questions:

  - What are the major mRNA regulatory pathways affected by GLRaV/5 during the symptomatic period and how it does affect the overall regulatory network and the resultant metabolites?

  - Is there an effect of the virus on the synchronicity of the transcriptional program (Gouthu et al., 2014)?

**Experimental design**

- Healthy plant
- Infected plant
- Green/Red berries
- Healthy/Infected within berries
- Healthy/Infected within stage

- High definition mass spectrometry - Unbiased metabolomics

- The red stage of RO tends to the most affected stages by the virus infection

- The viral infection tends to reorganize the transcriptional program of the berry.
3D clustering analysis (Gene-Condition-Time) using the (HP/IP) green berry experiment from comparison #1:

Examples of co-expression patterns

<table>
<thead>
<tr>
<th>Pattern</th>
<th>HP</th>
<th>IP</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>D</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>E</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>F</td>
<td>18</td>
<td>21</td>
</tr>
</tbody>
</table>

Interpretation:

- **Pattern A**: Co-expression patterns are reorganized in HP and IP (51%)
- **Pattern B**: Loss of the co-expression patterns in IP (12%)
- **Pattern C**: Induced co-expression patterns in IP (2%)
- **Pattern D**: Conserved co-expression patterns in HP and IP (16%)
- **Pattern E**: Constant patterns in HP and IP (19%)

**Gene Ontology Enrichment Analysis (AMIGO2 - REVIGO - Cytoscape):**

Remodeled co-expression patterns in HP and IP (51%)

- **Molecular Function (MF):**
  - Transcription activity
  - Acyl/Aminoacyl groups
  - Tetrapyrrole Binding

- **Cellular Component (CC):**
  - Chloroplast
  - Photosystem
  - Polysaccharide/glucosaminoglycan metabolism

**Exploring Correlation-based Network using graph theory properties:**

- Evaluating the influence of the virus on the overall topology of the transcriptional network
  - HP network: |r| > 0.9
  - IP network: |r| > 0.9

- The transcriptional network in IP is reorganized in a non-randomized way with the loss of "hubs" and the emergence of a new set of "hubs"

**Gene Ontology Enrichment Analysis (AMIGO2 - REVIGO - Cytoscape):**

Remodeled co-expression patterns in HP and IP (51%)

**Use of the betweenness centrality as an indicator of a node's centrality in a given network.**

- **Determine if the virus affects this parameter and therefore the topology of the network.**

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<th>Small RNA pipeline:</th>
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<td>(all small RNA libraries)</td>
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**Small RNAs landscape:**

- **miRNA target prediction:**
  - PamREAP (modified for plants, courtesy of Jixian Pamela Green)

**miRNA predictions:**

- Filtering Step #1: 1. stringent RNA 2. imperfect alignments 3. > 20 alignments
- Filtering Step #2: 1. structural RNA 2. strand bias > 0.9 Abundance Bias > 0.7
- Filtering Step #3: 26 nt imperfect alignments

**miRNA curated:**

- miRNA target prediction
  - Origami (new miRNA in data)
  - Strand Bias > 0.9

**miRNA target prediction:**

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- UEA sRNA hairpin

- 630 predicted miRNA precursors; 52 known miRNA precursors

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**miRNA curated:**

- 1/19/2016

If you need more information, visit poster 1129

**Overall main network properties for HP**
- Diameter: 13
- Density: 0.015
- Edges: 3,697
- Nodes: 457

**Overall main network properties for IP**
- Diameter: 13
- Cluster Coefficient: 0.34
- Edges: 4,651
- Nodes: 428

**Top 10 in HP**
- 1. Zeatin riboside
- 2. Zeatin
- 3. Glucosinolate
- 4. Glucosinolate precursor
- 5. Glucosinolate
- 6. Zeatin riboside
- 7. Zeatin
- 8. Glucosinolate precursor
- 9. Glucosinolate
- 10. Zeatin

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**Other major affected and non affected co-expression patterns in HP and IP (43%):**

- **BP**: Enrichment in phosphate metabolism, carbohydrates, monosaccharide metabolism
- **MF**: Enrichment in transcription factor activity
- **CC**: Enrichment in mostly chloroplast and Thylakoid part

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**Betweenness centrality**

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miRNAs-mRNAs inferred interactive in the correlation-based transcriptional network:

Overall Network in HP

First neighbors of 2 potential miRNA targets

Overall Network in IP

Knuckle-Protein (15)

SPL6 (104)

SPL6 (21)

Knuckle-Protein (79)

Conclusions:
• The effect of the virus on the transcriptome is berry stage specific.
• The effect of the virus on the reduction of the intracluster variability is marginal.
• 2/3 of the transcriptome DE during the ripening is re-routed to a different direction.
• Viral infection strengthens the transcriptional network in conjunction with changes in the nature of the major "hubs" responsible for the overall topology of the network.
• Induction of some miRNA targets affect the network topology by affecting the connectivity of "hubs" to their neighbors.

Perspectives:
• Improve and explore the use of graph-theory to identify relevant miRNA-mRNA interactors and develop new hypothesis with some functional validation experiments.
• Experimental step to confirm the Small RNA landscape.
• Run the "metabolome" side of the project in order to integrate these different layers of biological information.

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