Introduction

- In 2015, combined fresh and frozen blueberry (Vaccinium corymbosum) sales were at the number 2 position for berry sales in the US, with a market value of over $1.6 billion.
- Mummy berry disease, caused by the fungal pathogen Monilinia vaccinii-corymbosi, is one of the most economically important diseases of blueberries. If left untreated, crop damage as high as 50% can occur during normal growing seasons.
- Next-Generation Sequencing (NGS) is a fast and effective tool for gene expression studies.
- RNA-Seq was used in this study for identifying genes potentially involved in the mummy berry disease infection process.
- This study will help to fill some of the molecular informational gap for this disease.

Objectives

- Use NGS technology for gene expression analysis in response to mummy berry disease infection.
- Do a comparative analysis between infected and noninfected tissues of the same subject.
- Identify candidate genes for resistance to mummy berry disease in blueberry (Vaccinium sp.).

Assembly Stats: Monilinia vaccinii-corymbosi

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assembled from 454 genome sequence data using automatic word and bubble size 31</td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>200 bp</td>
</tr>
<tr>
<td>Maximum</td>
<td>141320 bp</td>
</tr>
<tr>
<td>Average</td>
<td>734 bp</td>
</tr>
<tr>
<td>Contig Count</td>
<td>836 bp</td>
</tr>
<tr>
<td>N50</td>
<td>1017 bp</td>
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</table>

Assembly Stats: Blueberry cv. Arlen

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Length</th>
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<tbody>
<tr>
<td>Assembled from Illumina transcriptome sequence data using bubble size 150 and word size 31</td>
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<tr>
<td>Minimum</td>
<td>200 bp</td>
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<tr>
<td>Maximum</td>
<td>124040 bp</td>
</tr>
<tr>
<td>Average</td>
<td>869 bp</td>
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<tr>
<td>Contig Count</td>
<td>638 bp</td>
</tr>
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</table>

Methods and Materials

- Samples were frozen in liquid N in the field and transferred to -80°C freezer on dry ice.
- RNA was extracted from 16 tissues (12 unique) from susceptible cv. Arlen using the Sigma Mummy service.
- cDNA libraries were created using the KAPA mRNA Stranded Seq kit with a target insert size of 200-300 bp.
- Biocentric NEXTflex DNA barcodes were used for multiplexing samples.
- Libraries were run at the NCSU Genomic Sciences Lab using the Illumina MiSeq 150bp SR (v3) service.
- CLC Genomics WB (v9.5) was used for QC, RNA-Seq Analysis, and transcriptome assembly.
- Roche 454 genomic reads were obtained for Monilinia vaccinii-corymbosi from Dr. Marc Cubeta and Kathleen Burchardt and a genomic assembly was made using CLC Genomics WB.
- BLASTX 2.4.0+ was performed for the Arlen transcriptome assembly contigs using a local server.
- KEGG Pathway Mapping was performed in Blast2GO v 4.0.7 on the entire transcriptome assembly for Arlen.
- Genes of interest were filtered from the table for the t-test performed in CLC using a threshold p-value of ≤ 0.05 and a threshold fold change with absolute value > 3.99.

Table 1. Assembly statistics for transcriptome assembly of cv. Arlen and gene expression studies of Monilinia vaccinii-corymbosi fungal pathogen.

<table>
<thead>
<tr>
<th>Arlen Contig</th>
<th>t-test: Fold change</th>
<th>t-test: P-value</th>
<th>Description</th>
<th>e-value</th>
<th>Sim Mean</th>
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<tbody>
<tr>
<td>22</td>
<td>-13.3914</td>
<td>0.02913</td>
<td>jasmonic acid-amido synthetase</td>
<td>0</td>
<td>88.00%</td>
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<tr>
<td>6382</td>
<td>-143.829</td>
<td>0.00026</td>
<td>ethylene response sensor 1</td>
<td>Vitis vinifera</td>
<td>0</td>
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<tr>
<td>1890</td>
<td>-80.7437</td>
<td>0.00013</td>
<td>phyto-responsive small GTP-binding</td>
<td>Solanum lycopersicum</td>
<td>1.1E-121</td>
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<tr>
<td>10296</td>
<td>-31.9057</td>
<td>0.00036</td>
<td>Disease resistance</td>
<td>Theobroma cacao</td>
<td>6.6E-113</td>
</tr>
<tr>
<td>61024</td>
<td>1000000</td>
<td>1.26E-06</td>
<td>CMLSP PK3 kinase</td>
<td>1.4E-105</td>
<td>98.15%</td>
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<tr>
<td>11849</td>
<td>-21.3933</td>
<td>0.00049</td>
<td>pathogenesis-related 5-like</td>
<td>2.8E-46</td>
<td>75.30%</td>
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<tr>
<td>8474</td>
<td>4.710895</td>
<td>0.00964</td>
<td>Pathogenesis-related</td>
<td>Theobroma cacao</td>
<td>4.1E-78</td>
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<tr>
<td>22085</td>
<td>-5.14772</td>
<td>0.00487</td>
<td>Disease resistance RPM1-like</td>
<td>Vitis vinifera</td>
<td>5.2E-71</td>
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<tr>
<td>44536</td>
<td>1000000</td>
<td>0.03932</td>
<td>salicylate hydroxylase</td>
<td>1.9E-66</td>
<td>86.50%</td>
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<tr>
<td>1425</td>
<td>-1000000</td>
<td>0.0215</td>
<td>Resistance partial</td>
<td>4.5E-41</td>
<td>77.15%</td>
</tr>
</tbody>
</table>

Table 2. List of 10 candidate genes that are up or down in the early stages of infection.

References


Results

- Out of an assembly of 48349 contigs total for cv. Arlen, 413 genes were selected for further analysis.
- These 413 genes fit within the parameters for p-value and fold-change for the t-test and their hit descriptions or GO names listed contained key words that were chosen based on previous studies.
- Key words include: Jasmonic acid, Salicylic acid, Ethylene, Pathogenesis, Biotic stress, Defense response, Resistance, etc.
- Several fungal genes were found in the cv. Arlen transcriptome assembly, indicating that we still have fungal read contamination of our blueberry sequence data.

Conclusion

- Based on the hit descriptions of our 413 selected genes, this data may likely contain promising results for candidate genes for resistance to mummy berry disease.
- The 413 genes found thus far in this study will need to be further filtered in order to further narrow down our list of potential candidate genes for resistance.
- Future work will focus on the addition of more replications and other cultivars along the resistance/susceptibility spectrum for comparison.
- More genomic data is also needed for the Monilinia corymbosi pathogen, due to the continued contamination of our blueberry transcriptome data with fungal sequences. This will allow us to filter fungal sequences out of our blueberry data.