Next generation sequencing of rabbiteye blueberry (*Vaccinium virginatum* ‘Premier’) and transcriptome comparisons to blueberry genomic resources

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**Abstract**

*Vaccinium virginatum* (syn. *V. ashei*) is commonly known as rabbiteye blueberry and native to the Southeastern United States. Cultivars are typically grown from North Carolina south to Florida and west to Texas for commercial blueberry production. In the Southeast, plants exhibit superior environmental tolerance and have fewer disease and insect concerns that highbush varieties (*Vaccinium corymbosum*), although some southern highbush (*Vaccinium corymbosum* × *Vaccinium darrowii*) include *V. virginatum* in their genetic backgrounds. Extensive genomic work has been done on *V. corymbosum*, both diploid and tetraploid, but not much has been done with *V. virginatum*, which is hexaploid. Our study included five *V. virginatum* cultivars; TifBlue, Climax, PowderBlue, Austin, and Premier. Tissues were collected and flash frozen in liquid nitrogen from clonally propagated plants for each cultivar at multiple developmental stages including buds, berries, leaves, and roots. Tissue was also collected from root and leaves during drought treatments. First sequencing results were produced for several growth stages of ‘Premier’ using Nextera Total RNA kit and Illumina MiSeq instruments. Transcriptomes were compared to existing genomic resources for highbush.

**Rabbiteye Blueberry**

*Vaccinium virginatum*

syn. *V. ashei*

2n=6x=72, hexaploid

Natural populations across Gulf coast with morphological variability between populations.

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**Preiliminary Transcriptome Results**

**TruSeq Total RNA kit, Plant RiboZero, MiSeq ver. 3, 75 bp**

<table>
<thead>
<tr>
<th></th>
<th>reads</th>
<th>assembled</th>
<th>contigs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bud, pink tip</strong></td>
<td>71,152,832</td>
<td>52,382,793</td>
<td>58,585</td>
</tr>
<tr>
<td><strong>Leaf, emerging</strong></td>
<td>53,510,106</td>
<td>45,345,416</td>
<td>44,565</td>
</tr>
<tr>
<td><strong>Berry, green</strong></td>
<td>66,122,430</td>
<td>53,725,915</td>
<td>47,209</td>
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</tbody>
</table>

**Blast2Go** annotation is ongoing as we add more tissues to the data for ‘Premier’. Preliminary results show equivalent distribution of high quality hits in molecular, cellular, and biological functions when comparing the three tissues. Average contig size was small, roughly 400 to 500 bp, but functional annotation appears to be robust for the environmental tolerance traits that are most important to our expanded rabbiteye genetics project.

**Tissues that were collected from *Vaccinium virginatum* ‘Premier’ for transcriptome analyses.**

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**RNAseq**

**Can we use the diploid *V. corymbosum* reference genome for RNAseq studies using rabbiteyee?**

The Blueberry Reference Genome, 2008 SCRI grant, team led by Dr. Jeannine Rowland, USDA-ARS, “Generating Genomic Tools for Blueberry Improvement”.

- Diploid highbush (*V. corymbosum* W85-20)
- Draft genome from Dr. Allen Brown, NCSU (May 2013)

Tophat2 to align the blueberry reads onto the blueberry draft genome assembly. Looking for fragments that overlap annotated genes in the diploid reference.

**Coverage was low.** Altogether, we detected expression for 43.8% of blueberry genes. This could be due to differences between the diploid *V. corymbosum* and hexaploid *V. virginatum* rabbiteye, possible quality issues with the libraries, or incomplete reference genome.

**Numbers of genes with RPKM >= 1 for each sample type:**

- Curled leaf: 21,447
- Green berry: 21,195
- Pink tip: 25,171

There were 27,982 genes with expression level of 1 RPKM or higher.

**Results were comparable** to RNAseq analyses using data from ‘O’Neil’, which is a southern highbush with *V. virginatum* in its background (Wolcott × Florida 64-15, or 2.5% *V. darrowii*, 3.8% *V. virginatum*, and 10.5% *V. angustifolium*).

**Differential Expression for tissues under different environmental stress**

**Using diploid reference genome or de novo transcriptome assembly described here.**

We are most interested in gene expression profiles between tissues under environmental stress conditions. To that end, we have collected and flash frozen tissue from five rabbiteye cultivars (TifBlue, Climax, PowderBlue, Austin, and Premier) before, during, and after drought stress.

**pH Tolerance**

Roots secrete ions to change rhizosphere/soil pH and enhance mineral uptake?

**Drought Tolerance**

Faster stress detection, more responsive stomatal controls?

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