ABSTRACT
Since the early 1900s, the black raspberry (Rubus occidentalis L.) industry in the United States has steadily declined due to lack of adapted and disease resistant cultivars. Renewed interest in production and breeding new cultivars has been fueled by research into the use of their anthocyanin compounds as potential chemopreventative agents for certain cancers. The USDA-ARS NCGR manages and maintains a collection of over 175 black raspberry germplasm accessions, which includes newly collected wild accessions from 130 locations across 27 US states and two Canadian provinces. Evaluation of this wild germplasm led to the identification of four potential sources of aphid resistance, and potential new sources of resistance to the fungal pathogen Verticillium. We are developing the genomic infrastructure for black raspberry by developing, and making available, genomic tools including molecular markers for construction of linkage and physical maps, and a draft genome assembly that will benefit both black and red raspberry breeding programs across the U.S. To date, we have identified 42 SSR markers polymorphic in the parents of two elite crosses; 704 Gbp of sequence have been generated from six cDNA libraries of five tissues types of ‘Jewel’; and an initial genome assembly of 300Mbp has been generated from a highly homozygous accession. These developing genomic resources will be instrumental in building the infrastructure needed for identification of candidate genes or markers responsible for many traits of interest for development of improved black raspberry cultivars, and will inform decisions regarding germplasm value and usage, crossing, and selection through marker-assisted breeding.

Project Objectives
- Develop molecular markers in black raspberry for construction of genetic and physical maps
- Sequence and assemble the genome of highly homozygous black raspberry clone, ORUS 4115-3, collected from Rich Mountain, SC
- Develop transcriptome data for a variety of tissue types
- Identify QTL associated with traits of interest

Background & Introduction
- Native to North America
- Lack of variability & disease resistance in elite germplasm
- Only 4 new cultivars since 1975
- ‘Munger’, most of acreage
- Wild germplasm collected in 2007: - New sources of aphid resistance identified
- - Introgressed in ORUS 4304 and 4305

Fig. 1. Source plant of ORUS 4115-3 (A) and fruits (B) when collected in 2007 in SC

Segregating Populations

ORUS 4305 (115 progeny)
ORUS 3021 x ORUS 4153-1

ORUS 4304 (191 progeny)
ORUS 4158-2 x ORUS 3021-2

110,127 polymorphic black raspberry SSRs (Ro) to map in ORUS 4305, 4304
96, 99 red raspberry SSRs (Rh + Bristol) to map in ORUS 4305, 4304

Transcriptome

Remaining:
- Verticillium-infected roots
- Clean roots
- Canes in Spring
- Jewel plants inoculated with Verticillium, 7/19/2012
- Roots harvested 9/13/2012

Preliminary Genome Assembly
Initial assembly of the ORUS 4115-3 genome is based on Illumina HiSeq paired-end and mate-pair sequencing. Total genome coverage depth was ~347x, assuming an estimated genome size of 300 Mbp.

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<thead>
<tr>
<th>Table 1. Genome assembly statistics</th>
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<tbody>
<tr>
<td>Assembly (Mbp)</td>
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<tr>
<td>Number of scaffolds</td>
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<tr>
<td>Average scaffold length (bp)</td>
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<td>N50 (bp)</td>
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<td>N90 (bp)</td>
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<td>Percent Ns</td>
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<tr>
<td>Percent SNP bases</td>
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<td>Percent assembled reads</td>
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<td>Number of genes (Augustus)</td>
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Results
- Low percent SNP bases (0.06%) confirms very low heterozygosity
- High percent of read assembly is consistent with high quality sequence data

Next
- Generate RNA-seq data from Verticillium-infected and clean roots, canes in Spring
- Use RNA-seq data to improve gene models

Future Work
- Complete genome assembly and transcriptome sequencing
- Construct well-saturated linkage maps for black raspberry
- Study genotype by environment interaction in the two crosses
- Identify QTL using SSRs and genome-wide SNP markers and map traits of interest
- Comparative genomics in Rubus and Rosaceae

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