Gene expression profiling of resistance and susceptibility to Beet curly top virus in sugarbeet.

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Beet Curly Top disease (BCT)

- Viral disease; dry and hot areas (Western USA, Middle East)
- Transmitted by Beet leafhopper; *Circulifer tenellus*
- Three virus strains Cal/Logan, Worland, & Severe
- Seed trt. insecticide; Poncho-Beta, Cruiser, NipsIT

Available knowledge

- There are three levels of phenotypic symptoms directly related to the 3 virus strains
- The symptoms define a quantitative trait inheritance
- The current diagnostics (PCR-based) depend on only 3 clones; one from each strain
- The natural leafhopper populations carry the three strains
- No knowledge of effect of strains interaction on symptoms

Objectives:

- To identify genes regulating resistance to Beet Curly Top
- To develop allele specific DNA markers for marker assisted breeding.
- Introgress resistance genes into public germplasm

Approaches

- Genetic linkage mapping (requires RILs)
- LD analysis and association mapping
- Gene expression analysis (RNA Sequencing):
  - Resistant doubled haploid line
  - Homozygous susceptible inbred line
  - *NIL will be ideal for this approach*
Materials and Methods

- Control population: Non-infectious (NI) leafhopper colony; provided by W. Wintermantel (Salinas, CA)
- Identify plant(s) that carry single strain or combinations of strains (PCR) and encage NI hoppers to feed on these plants
- Isolate leafhopper colonies that carry 1 or combinations of 2 or 3 strains
- Use homozygous resistant (KDH13) and susceptible (K19-19) genotypes

Plants infection and RNA sequencing

- Plants grown to 4 leaf stage in a Conviron® growth chamber
- Plants placed in cages with 20-30 leafhoppers
- Total RNA isolated from leaves 5 Days Post Infection
- 24 libraries (8trt. X3reps) were constructed using:
  - Illumina TruSeq - Stranded - Ribo-Zero kit
  - 24 lib. pooled and sequenced in 6 lanes in Illumina HiSeq2500v4

Treatments conditions and symptoms

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LT</th>
<th>NI</th>
<th>BC</th>
<th>BM</th>
<th>BCM</th>
<th>BCS</th>
<th>BCMS</th>
<th>BMSS</th>
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<tbody>
<tr>
<td>Genotype</td>
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<td>KDH13</td>
<td>K19-19</td>
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<tr>
<td>Hoppers</td>
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<td>NI</td>
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<tr>
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Genotypes X Virus-Vector matrix

Data Analysis

- Software used:
  - FastQC, Bowtie, BWA, Tophat, Cufflinks, Samtools
- Total paired trimmed reads per sample ranged from 73Mi to 200Mi reads
- Reference genome assemblies (alignment):
  - RefBeet-1.1 & 1.2 http://bseq.molgen.mpg.de
- BCTV genome

28 Pair-wise comparisons (Cuffdiff)

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Generated 56 tables of thousands of significantly differentially expressed genes/transcripts.
Differentially expressed genes (DEGs)

- Based on FDR (false discovery rate-adjusted p-value) cut-off of <0.01
- Up or down regulated genes due to hoppers feeding.
- Up or down based on single or a combination of two or three strains.

DEGs Susceptible (K19-19) vs. Resistant (KDH13)

- UP: BCMSS vs. BCMS
- Down: BCMSS vs. BCMS

IDH1 : KDH13

Isocitrate dehydrogenase-gene subunit (7.8Kb transcript in Chrom:1)

DEGs: Insect-feeding effect

- Down: NI vs. LT
- UP: NI vs. LT

AtL6 : KDH13 vs. K19-19

Ubiquitin ligase gene subunit (1.4Kb transcript in chrom:6)

Essential for Pseudomonas defense response in Tomato. Transgenic plants over expressed AtL6 and AtL31 showed increased resistance and knockout showed reduced resistance to Pseudomonas

Genes detected only in K19-19

Genes detected only in KDH13
Summary

- Identified differentially expressed genes due to insect feeding
- Identified differentially expressed genes due to single strain
- Highly expressed genes/transcripts undetectable in resistant line (resistance genes)
- Highly expressed genes/transcripts undetectable in susceptible line (susceptibility)