Variation in the flax genome and its relation to bioproducts

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Outline:
Types of variation
• gene expression (Fusarium)
• transposable elements
• SNPs (spontaneous & induced)

Infection with Fusarium oxysporum
• CDC Bethune vs. Lutea
• Fol isolates #65, #81 (K. Rashid)
• RNASeq 4 time points

Fusarium oxysporum RNASeq
Sampled 2, 4, 8, 18 days post inoculation

control
inoculated
**Fusarium oxysporum** RNASeq
CDC Bethune sampled 2, 4, 8, 18 days post inoculation

<table>
<thead>
<tr>
<th>Days post inoculation</th>
<th>Number of transcripts with expression</th>
<th>Number of differentially expressed transcripts (q&lt;0.05)</th>
<th>upregulated (q&lt;0.05)</th>
<th>downregulated (q&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>18,764</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>18,302</td>
<td>103</td>
<td>48</td>
<td>55</td>
</tr>
<tr>
<td>8</td>
<td>18,437</td>
<td>125</td>
<td>79</td>
<td>46</td>
</tr>
<tr>
<td>18</td>
<td>18,636</td>
<td>1043</td>
<td>1008</td>
<td>35</td>
</tr>
</tbody>
</table>

q values are corrected for multiple testing (Benjamini-Hockberg).

~ 50,000 transcripts including gene models and novel transcripts detected.

Day 18 pectin-related transcript enrichment

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<th>PG, PGIP, PLL, RGL</th>
<th>PME, PMEI, PAE</th>
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<td><img src="graph1.png" alt="" /></td>
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Day 18 pectin-related transcript enrichment significant at q<0.05

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Types of mobile DNA

- retrotransposon
- transposition

- Copia
- Mutator
- Gypsy

http://ramin.iut.ac.ir/yang-cycle-ethylene-biosynthesis


Key genes regulated in flavonoid/anthocyanin and carotenoid synthesis
- • Chalcone synthase
- • Dihydroflavonol reductase

Transformation of flax increases resistance to fusarium
- • Seven anthocyanidin synthases with log2-fold change > 6
- • Phytoene synthase

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Aquaporins and amino acid transporters allow better hyphal penetration and provide nutrients for the fungus

Expansins and cell wall lyases are usually activated during growth but weaken the cell wall which would allow easier pathogen entrance

Indole-3-acetic acid amino acid/amido hydrolases produce hormonally active IAA contributing to plant growth partly by regulating cell wall genes

Major latex proteins function is still unclear but they are usually upregulated upon pathogen infection, whereas here they are mostly repressed.
Distribution of mobile DNA in flax

Copia elements have been most active in recent flax evolution

Copia families in flax

Genomic copy number of Copia families measured by qPCR in selected flax cultivars
ANOVA among the cultivars for each TE family was followed by Tukey multiple comparison tests (*p* < 0.05).

Transcript abundance of Copia families measured by qRT-PCR in selected flax cultivars

Accession relationships according to transposon insertions

Locations of polymorphic Copia insertions

- 99/140 polymorphic bands sequenced
- 66/99 non-redundant insertion sites.
- 21.2% of interrupted exons.
- 45.5% of interrupted introns.
- 16.7% within 1kb of ORF
- 16.7% intergenic.

Copia insertion polymorphisms (examples)

Impact of Copia insertion on gene transcription
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- qRT-PCR
- 4 genes
- 5 varieties with/without Copia
- 3 tissues

Impact of Copia insertion on gene transcription

TE (Copia) summary

- Copy number varies between families, but not between cultivars
- Tissue-specific TE expression varies between cultivars
- Many examples of gene-associated insertions polymorphic between cultivars
- But ... gene-associated insertions have little effect on gene expression

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Examples of predicted large effect SNPs

Polymorphisms (SNPs) in re-sequenced varieties

(compared to CDC Bethune)
Reverse genetics
Find a phenotype by looking for a mutation in a known gene

Reverse genetics: three approaches

AMP1CON sequencing
PCR amplify 250bp target regions from barcoded pools of mutant DNA
~5 genes, 768 individuals per sequencing run
sequence on IonTorrent

Exome capture
capture ~250bp target regions using custom oligos (Comai & Henry)
~40,000 genes, <24 individuals per seq run
sequence on Illumina

Whole genome re-sequencing
no complexity reduction
>40,000 genes, 1 individual per lane
sequence on Illumina

EMS population exome capture results:
Average number of genes mutated per line:

20 mutations, Mb ~7500/gene

73 genes: very deleterious
• non-sense, splice-site and frameshift mutations

349 genes: deleterious
• non-synonymous mutations 0.05 > SIFT score

68 genes: weakly deleterious
• 0.05 < SIFT score < 0.01

3,054 mutations: neutral
• non-synonymous with SIFT score > 0.1,
• synonymous, introns, intergenic

Gene knockouts currently being validated
• differentially expressed in Fusarium experiment (disease resistance)
• differentially expressed in shoot apex (fiber identity)
• other cell wall and bioproduct related genes
Gene knockouts currently being validated

- disease resistance
  - pectin methylesterase (PME3), subtilisin, cinnamyl alcohol dehydrogenase (CAD), ornithine decarboxylase (OrnDC), terpene synthase, UDP-glycosyl transferase, many DUFs,
- fiber identity
  - PME inhibitor, IAA-induced4, transcription factors (bHLH, NAC), many DUFs...
- bioproducts
  - beta-galactosides (BGAL), trichome birefringence-like (TBL), hydroxynitrile lyase (HNL)

Acknowledgements

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